3. HEALTH EFFECTS

3.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of atrazine. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

3.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure (inhalation, oral, and dermal) and then by health effect (death, systemic, immunological, neurological, reproductive, developmental, genotoxic, and carcinogenic effects). These data are discussed in terms of three exposure periods: acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in tables and illustrated in figures. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELS have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not

the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals (LOAELs) or exposure levels below which no adverse effects (NOAELs) have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

3.2.1 Inhalation Exposure

No studies were located regarding cardiovascular, musculoskeletal, hepatic, renal, or dermal/ocular effects in humans or animals after inhalation exposure to atrazine.

No studies were located regarding the following effects in humans and/or animals after inhalation exposure to atrazine:

3.2.1.1 Death

3.2.1.2 Systemic Effects

3.2.1.3 Immunological and Lymphoreticular Effects

3.2.1.4 Neurological Effects

3.2.1.5 Reproductive Effects

Results of surveys of farm couples in Ontario, Canada, to assess reproductive effects of pesticides indicated weak associations between atrazine use on crops and in the yard with an increase in preterm delivery and with miscarriage (Savitz et al. 1997). Other surveys of Ontario farm couples indicated that atrazine was not associated with any decrease in fecundity as a result of effects on spermatogenesis (Curtis et al. 1999). In both of these cohort studies, it is probable that both dermal and inhalation exposure occurred (for additional study details, see Section 3.2.3.5 Dermal Reproductive Effects).

No studies were located regarding reproductive effects in animals after inhalation exposure to atrazine.

3.2.1.6 Developmental Effects

No studies were located regarding developmental effects in humans and/or animals after inhalation exposure to atrazine.

3.2.1.7 Cancer

No studies were located regarding cancer in humans and/or animals after inhalation exposure to atrazine.

3.2.2 Oral Exposure

Only one case report was located regarding effects from oral exposure to atrazine in humans (Pommery et al. 1993).

3.2.2.1 Death

The available information on the lethality of atrazine in humans is limited to a case report of a man intentionally ingesting 500 mL weed killer containing 100 g of atrazine, 25 g of aminotriazole, 25 g of ethylene glycol, and 0.15 g of formaldehyde (Pommery et al. 1993); the approximate atrazine ingested was 1,429 mg/kg. The man exhibited coma, circulatory collapse, metabolic acidosis, and gastric bleeding and died 3 days later. The study authors stated that some of the symptoms displayed by the patient upon hospital admission (metabolic acidosis and large anion gap) indicated that ethylene glycol was an important toxicant. Ethylene glycol was present in the blood (300 mg/L), and formic and oxalic acids were detected in the urine. The study authors also speculated that aminotriazole and possibly formaldehyde, as well as atrazine, may have contributed to the symptoms and ultimate outcome of the case.

Atrazine has a low acute toxicity in laboratory animals. Exposure of pregnant Charles River rats to 700 mg/kg/day atrazine in the commercial product Aatrex throughout gestation resulted in 78% mortality; the cause of death was not determined (Infurna et al. 1988). Acute oral LD_{50} values for adult male and female rats of 1,471 and 1,212 mg/kg (Ugazio et al. 1991b) and 737 and 672 mg/kg (Gaines and Linder 1986), respectively, have been reported. An LD_{50} of 2,310 mg/kg was reported for young (weanling)

male rats (Gaines and Linder 1986), indicating a lower sensitivity to atrazine than adult rats. A 15% increase in mortality was observed in female Sprague-Dawley rats exposed to 39.2 mg/kg/day atrazine for up to 24 months (Wetzel et al. 1994); mortality was not affected in similarly exposed female Fischer-344 rats (Wetzel et al. 1994).

Cattle that accidently consumed an unknown quantity of spilled Aatrex (containing 76% atrazine) became ill and one became recumbent and died within 8 hours (Jowett et al. 1986). Necropsy results revealed edematous lungs and a froth in the trachea. Six other cattle died within 3 days after exhibiting anorexia, salivation, tenesmus, stiff gait, and weakness.

3.2.2.2 Systemic Effects

No studies were located regarding systemic effects in humans after oral exposure to atrazine. The highest NOAEL values and all LOAEL values from each reliable study for the systemic effects of atrazine in each species and duration category are recorded in Tables 3-1 and 3-2, and plotted in Figure 3-1. These studies are discussed below.

Respiratory Effects. No animal studies were located that evaluated respiratory function. Mice gavaged with a single dose of 875 mg/kg atrazine (Fournier et al. 1992), sheep that consumed hay sprayed with atrazine (approximately 47 mg atrazine/kg body weight/day) for 25 days (Johnson et al. 1972), and pigs treated with 2 mg/kg/day atrazine in the feed for 19 days (, uri$f$ et al. 1999) had no gross or histopathological lesions of the lungs. Chronic exposure of male and female rats to up to 52 and 70.6 mg/kg/day atrazine, respectively, in the diet also had no gross or histopathological lung lesions (EPA 1984a, 1987).

Cardiovascular Effects. Alterations in electrocardiograph measures and heart pathology were observed in dogs exposed to about 34 mg/kg/day in the diet for 52 weeks (EPA 1989). Observed electrocardiographic changes consisted of slight to moderate increases in heart rate (primarily in males), moderate decreases in P-II values in both sexes, moderate decreases in PR values, slight decreases in QT values, atrial premature complexes in one female, and atrial fibrillation in both sexes. Gross postmortem examination revealed moderate to severe dilatation of right and/or left atria in the majority of animals, and some dogs had fluid-filled pericardium and enlarged heart. Atrophy and myolysis of atrial myocardium and edema of the heart were also observed in these dogs. No cardiac abnormalities were observed at 5 mg/kg/day. These cardiac effects are supported by the finding of degeneration of a small

Table 3-1. Levels of Significant Exposure to Atrazine - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	ACUTE EXPOSURE						
	Death						
1	Rat (Sherman)	1x (GO)				737 M (adult LD_{50}) 672 F (adult LD_{50}) 2310 M (weanling LD_{50})	Gaines and Linder 1986 technical grade
2	Rat (Sprague-Dawley)	Gd 6-15 1x/d (GW)				700 (78% pregnant females died)	Infurna et al. 1988 Aatrex
3	Rat (NS)	1x (GW)				1471 M (LD_{50}) 1212[b] F (LD_{50})	Ugazio et al. 1991b Fogard 45% atrazine and purified
	Systemic						
4	Rat (Fischer- 344)	7d 1x/d (GO)	Endocr	60	120 (increased pituitary weight; impaired testosterone metabolism in pituitary and hypothalamus)		Babic-Gojmerac et al. 1989 recrystallized
5	Rat (Long-Evans)	1x (GW)	Endocr	200	300 (decreased serum LH and prolactin)		Cooper et al. 2000 97.1% pure
			Bd Wt	300			

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
6	Rat (Sprague-Dawley)	1x (GW)	Endocr	300					Cooper et al. 2000 97.1% pure
			Bd Wt	300					
7	Rat (Long-Evans)	3d 1x/d (GW)	Endocr		50	(decreased serum LH and prolactin; increased pituitary prolactin)			Cooper et al. 2000 97.1% pure
			Bd Wt	300					
8	Rat (Sprague-Dawley)	3d 1x/d (GW)	Endocr	200	300	(decreased serum prolactin levels)			Cooper et al. 2000 97.1% pure
			Bd Wt	300					
9	Rat (Long-Evans)	1x (GW)	Endocr	300					Cooper et al. 2000 97.1% pure
10	Rat (Long-Evans)	3d 1x/d (GW)	Endocr		300	(effects on neuroendocrine regulation)			Cooper et al. 2000 97.1% pure
11	Rat (Holtzman)	Gd 1-8 (GW)	Endocr	50	100	(decreased serum progesterone and LH)			Cummings et al. 2000 97.1% pure
			Bd Wt				50	(43% decrease in body weight gain)	
12	Rat (Sprague-Dawley)	Gd 1-8 (GW)	Endocr		200	(increased serum estradiol)			Cummings et al. 2000 97.1% pure
			Bd Wt	50			100	(69% decrease in body weight gain)	

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
13	Rat (Long-Evans)	Gd 1-8 (GW)	Endocr	50	100	(decreased serum LH)			Cummings et al. 2000 97.1% pure
			Bd Wt				50	(57% decrease in body weight gain)	
14	Rat (Fischer- 344)	Gd 1-8 (GW)	Endocr	100	200	(decreased serum LH)			Cummings et al. 2000 97.1% pure
			Bd Wt	50			100	(74% decrease in body weight gain)	
15	Rat (Sprague-Dawley)	Gd 6-15 1x/d (GW)	Bd Wt	70			700	(severe body weight loss)	Infurna et al. 1988 Aatrex
16	Rat (Wistar)	6 or 12d 1x/d (GW)	Endocr		240	(decreased serum T3 and histological changes in the thyroid)			Kornilovskaya et al. 1996 95% pure
17	Rat (Fischer- 344)	12d every 48hr (GO)	Bd Wt	120					Peruzovic et al. 1995 purified
18	Rat (Wistar)	14d 1x/d (G)	Renal		100	(increased urinary sodium, potassium, chloride, and protein levels; increased serum LDH and HBDH activities)			Santa Maria et al. 1986 analytical grade

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
19	Rat (Wistar)	7 or 14d 1x/d (G)	Hepatic		100 (increased serum lipids, AP, and ALT)		Santa Maria et al. 1987 analytical grade
			Bd Wt		100 (25% decrease in body weight)		
20	Rat (Fischer- 344)	7d 1x/d (GO)	Endocr		120 M (increased pituitary weight)		Simic et al. 1994 >99% pure
			Bd Wt			120 F (45% decreased body weight gain)	
21	Rat (Wistar)	ppd 1-4 2x/d (G)	Endocr	12.5 F	25 F (decreased prolactin release in response to pup suckling)		Stoker et al. 1999 98% pure
22	Rabbit (New Zealand)	Gd 7-19 (GW)	Bd Wt	1[c]	5 (slight decrease in body weight gain)	75 (severe weight loss during exposure)	Infurna et al. 1988 Aatrex
	Neurological						
23	Rat (Fischer- 344)	12d every 48hr (GO)			120 (developmental neurobehavioral changes)		Peruzovic et al. 1995 purified
24	Rat (Wistar)	1x (GW)				100 (alteration of nerve stimulus conduction)	Podda et al. 1997 NS
	Reproductive						
25	Rat (Long-Evans)	1 or 3d (GW)		150		300 (altered estrus cyclicity)	Cooper et al. 2000 97.1% pure

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
26	Rat (Holtzman)	Gd 1-8 (GW)		50		100	(increased percent postimplantation loss, and decreased serum progesterone and serum LH)	Cummings et al. 2000 97.1% pure
27	Rat (Sprague-Dawley)	Gd 1-8 (GW)		200				Cummings et al. 2000 97.1% pure
28	Rat (Long-Evans)	Gd 1-8 (GW)		200				Cummings et al. 2000 97.1% pure
29	Rat (Fischer- 344)	Gd 1-8 (GW)		50		100	(increased percent preimplantation loss; decreased uterine weights)	Cummings et al. 2000 97.1% pure
30	Rat (Fischer- 344)	12d every 48hr (GO)		120				Peruzovic et al. 1995 purified
31	Rat (Fischer- 344)	7d 1x/d (GO)				120 F	(altered ovarian/estrus cyclicity; reduced fecundity)	Simic et al. 1994 >99% pure

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
	Developmental								
32	Rat (Sprague-Dawley)	Gd 6-15 1x/d (GW)		10	70	(incomplete ossification of skull, hyoid bone, teeth, forepaw metacarpals, and hindpaw distal phalanges)	700	(increased postimplantation loss/litter)	Infurna et al. 1988 Aatrex
33	Rat (Fischer- 344)	12d every 48hr (GO)			120	(neurobehavioral changes)			Peruzovic et al. 1995 purified
34	Rat (Wistar)	ppd 1-4, 6-9, or 11-14 2x/d (G)		12.5 M	25 M	(increased inflammation of lateral prostate, myeloperoxidase levels, and total DNA in prostate of male offspring)			Stoker et al. 1999 98% pure
35	Rabbit (New Zealand)	Gd 7-19 (GW)		5			75	(postimplantation losses, decreased fetal body weight, nonossification of forepaw metacarpals and middle phalanges, hindpaw talus and middle phalanges, and patella)	Infurna et al. 1988 Aatrex

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	INTERMEDIATE EXPOSURE						
	Systemic						
36	Rat (Sprague-Dawley)	28d 1x/d (GW)	Hepatic	5	50 (increased relative liver weight)		Aso et al. 2000 98.7% pure
			Renal	50			
			Endocr	50			
			Bd Wt	50			
37	Rat (Fischer- 344)	28d 1x/d (GW)	Hepatic	50			Aso et al. 2000 98.7% pure
			Renal	50			
			Endocr	50			
			Bd Wt	50			
38	Rat (Donryu)	28d 1x/d (GW)	Hepatic	5	50 (increased relative liver weight)		Aso et al. 2000 98.7% pure
			Renal	50			
			Endocr	50			
			Bd Wt	50			
39	Rat (Wistar)	6 or 12 mo 5 d/wk (F)	Bd Wt			2.7 (30% decreased body weight gain)	Cantemir et al. 1997 96% pure
40	Rat (Long-Evans)	21d 1x/d (GW)	Bd Wt	150	300 (about 10% decrease in body weight gain)		Cooper et al. 1996 >97.1% pure

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
41	Rat (Sprague-Dawley)	21d 1x/d (GW)	Bd Wt	300			Cooper et al. 1996 >97.1% pure
42	Rat (Long-Evans)	21d 1x/d (GW)	Endocr		75 (decreased serum LH; increased pituitary prolactin)		Cooper et al. 2000 97.1% pure
			Bd Wt	150	300 (decreased body weight gain)		
43	Rat (Sprague-Dawley)	21d 1x/d (GW)	Endocr		75 (increased pituitary prolactin)		Cooper et al. 2000 97.1% pure
			Bd Wt	150	300 (decreased body weight gain)		
44	Rat CFY	3mo (F)	Hemato	75			Desi 1983 technical purity
			Hepatic	75			
			Renal	38	75 (increased kidney weight)		
			Bd Wt		38 (decreased body weight gain)		
45	Rat (Sprague-Dawley)	14-23d 1x/d (GW)	Endocr		100 (increased adrenal weights; plasma estradiol levels decreased by 61%)		Eldridge et al. 1994b >96% pure
			Bd Wt		100 (body weight decreased by 16%)		

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
46	Rat (Fischer- 344)	14-23d 1x/d (GW)	Endocr		100 (increased adrenal weights)		Eldridge et al. 1994b >96% pure
			Bd Wt	100			
47	Rat (Wistar)	20d (ppd 22-41) (GW)	Hepatic	100	200 (decreased absolute and increased relative liver weights)		Laws et al. 2000 97.1% pure
			Renal	100	200 (decreased absolute and relative kidney weights)		
			Endocr		12.5 (decreased absolute and relative pituitary weight)		
			Bd Wt	100	200 (16% decrease in body weight gain)		
48	Rat (Fischer- 344)	1, 3, or 9 mo (F)	Endocr	45.2			Wetzel et al. 1994 97% pure
			Bd Wt		22.6 (body weight gain decreased by 11%)		
49	Rat (Sprague-Dawley)	1, 3, or 9 mo (F)	Endocr		6.9 (increased plasma estradiol levels)		Wetzel et al. 1994 97% pure
			Bd Wt		39.2 (body weight gain decreased by 15%)		

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
50	Pig (Landrace)	19d (F)	Resp	2			Curic et al. 1999 >99% pure
			Cardio		2 (degeneration of a small number of myocardial fibers)		
			Hepatic		2 (mild degeneration and inflammation and mild chronic interstitial hepatitis)		
			Renal			2 (subacute glomerulitis; degeneration and desquamation of proximal tubules)	
			Endocr	2			
51	Pig landrace	19d (F)	Hepatic		2 (350% increase in serum gamma-glutamyltransferase; mild liver histological changes)		Gojmerac et al. 1995 99% pure
	Immunological/Lymphoreticular						
52	Rat (Wistar)	3wk (F)			15.4 (lymphopenia)		Vos et al. 1983b 97% pure
53	Pig (Landrace)	19d (F)			2 (lymphoid depletion in lymph nodes and spleen)		Curic et al. 1999 >99% pure
	Neurological						
54	Rat CFY	3mo (F)		75			Desi 1983 technical purity

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)		LOAEL Serious (mg/kg/day)		Reference Chemical Form
	Reproductive								
55	Rat (Sprague-Dawley)	28d 1x/d (GW)		50					Aso et al. 2000 98.7% pure
56	Rat (Fischer- 344)	28d 1x/d (GW)		50					Aso et al. 2000 98.7% pure
57	Rat (Donryu)	28d 1x/d (GW)		50					Aso et al. 2000 98.7% pure
58	Rat (Long-Evans)	21d 1x/d (GW)		75			150	(disrupted estrus cycle; altered serum estradiol and progesterone levels)	Cooper et al. 1996 >97.1% pure
59	Rat (Sprague-Dawley)	21d 1x/d (GW)		75			150	(altered estrus cyclicity; elevated serum progesterone; pseudopregnancy)	Cooper et al. 1996 >97.1% pure
60	Rat (Sprague-Dawley)	14-23d 1x/d (GW)					100	(altered estrus cyclicity; decreased ovarian weights; decreased plasma estradiol levels)	Eldridge et al. 1994b >96% pure
61	Rat (Fischer- 344)	14-23d 1x/d (GW)			100	(decreased ovarian and uterine weights)	300	(altered estrus cyclicity)	Eldridge et al. 1994b >96% pure

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference Chemical Form
62	Rat (Sprague-Dawley)	45d 1x/d (GW)		5		40	(abnormal estrus cycle)	Eldridge et al. 1999a 97.1% pure
63	Rat (Sprague-Dawley)	26w 1x/d (F)		4.6		33	(abnormal estrus cycle)	Eldridge et al. 1999a 97.1% pure
64	Rat (Charles River)	2 gen (F)		26.7				EPA 1987b technical--% NS
65	Rat (Fischer- 344)	1, 3, or 9 mo (F)		45.2				Wetzel et al. 1994 97% pure
66	Rat (Sprague-Dawley)	1, 3, or 9 mo (F)				6.9	(increased length of estrus)	Wetzel et al. 1994 97% pure
67	Pig (Landrace)	19d (F)				2	(ovarian cysts; disruption of estrus cyclicity)	Curic et al. 1999 >99% pure
68	Pig landrace	19d (F)				2	(ovarian histopathology; disrupted estrogen and progesterone levels; anestrus)	Gojmerac et al. 1996 99% pure
69	Pig Swedish Landrace x Large Yorkshire	19d (F)				1	(altered serum estradiol concentrations; anestrus)	Gojmerac et al. 1999 NS

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
Developmental							
70	Rat Charles River)	2 gen (F)		30.9			EPA 1987b technical--% NS
71	Rat (Wistar)	20d (ppd 22-41) (GW)		25	50 (delayed vaginal opening)		Laws et al. 2000 97.1% pure
72	Rat (Wistar)	31d 1x/d (GW)			12.5 (delayed preputial separation)		Stoker et al. 2000 97.1% pure

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	CHRONIC EXPOSURE						
	Death						
73	Rat (Sprague-Dawley)	12, 15, 18, or 24 mo (F)				39.2 (15% increase in mortality)	Wetzel et al. 1994 97% pure
74	Dog (Beagle)	52wk (F)				33.80 F (death in 1/6 dogs)	EPA 1989 technical
	Systemic						
75	Rat (CD)	12mo (F)	Resp	52.0			EPA 1984a, 1987a technical--% NS
			Cardio	52.0			
			Gastro	52.0			
			Hemato	34.6 F	70.6 F (decreased RBC, hemoglobin, hematocrit; increased platelet, leukocyte, mean corpuscular hemoglobin)		
			Musc/skel	52.0			
			Hepatic	25.5 M	52 M (decreased liver weight, total triglyceride, globulin; increased albumin/globulin ratio)		
			Renal	25.5 M	52 M (decreased kidney weight, specific gravity; increased urine volume, pelvic calculi)		
			Endocr	25.5 M	70.6 F (increased adrenal gland weight; enlarged pituitaries)		
			Dermal	52.0 M			
			Ocular	52.0 M			
			Bd Wt	3.5 M	25 (decreased body weight)		
			Metab	25.5 M	52 (decreased serum glucose, calcium)		

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
76	Rat (Charles River)	2 gen (F)	Bd Wt	2.4	26.7 (10-15% decrease in body weight gain)		EPA 1987b technical--% NS
77	Rat (Fischer- 344)	126wk (F)	Bd Wt	29 M	58 M (10% decrease in body weight gain)		Pinter et al. 1990 98.9% pure
78	Rat (Fischer- 344)	12, 15, 18, or 24 mo (F)	Endocr	45.2			Wetzel et al. 1994 97% pure
79	Rat (Sprague-Dawley)	12, 15, 18, or 24 mo (F)	Endocr	39.2			Wetzel et al. 1994 97% pure

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
80	Dog (Beagle)	52wk (F)	Resp	33.80			EPA 1989 technical
			Cardio	4.97		33.65 (electrocardiographic changes; atrial dilatation; fluid-filled pericardium; enlarged heart; atrophy of atrial myocardium; edema)	
			Gastro	33.80			
			Hemato	4.97	33.65 (decreased RBC, hemoglobin, and hematocrit; increased platelet counts)		
			Musc/skel	33.80			
			Hepatic	4.97	33.65 M (increased relative liver weight; increased liver to brain weight)		
			Renal	33.80			
			Endocr	33.80			
			Dermal	33.80			
			Ocular	33.80			
			Bd Wt	4.97	33.65 M (body weight decreased by 19%)		
	Reproductive						
81	Rat (Fischer-344)	12, 15, 18, or 24 mo (F)		45.2			Wetzel et al. 1994 97% pure
82	Rat (Sprague-Dawley)	12, 15, 18, or 24 mo (F)				6.9 (increased length of estrus after 18 months)	Wetzel et al. 1994 97% pure

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference Chemical Form
	Cancer						
83	Rat (CD)	12mo (F)				70.6 F (CEL: increased incidence of mammary tumors and soft tissue tumors)	EPA 1984a, 1987a technical--% NS
84	Rat (CD)	24mo (F)				0.7 F (CEL: increased incidence of mammary tumors and soft tissue tumors)	EPA 1984a, 1987a technical--% NS
85	Rat (Fischer- 344)	126wk (F)		29	58 M (increased incidence of benign mammary gland tumors)		Pinter et al. 1990 98.9% pure
86	Rat (Fischer- 344)	126wk (F)				58[b] M (CEL: increased number of rats with malignant tumors) 65 F (CEL: increased incidence of uterine adenocarcinoma and leukemia/lymphoma; increased number of rats with malignant tumors)	Pinter et al. 1990 98.9% pure
87	Rat (Fischer- 344)	24 mo (F)		45.2			Wetzel et al. 1994 97% pure

Table 3-1. Levels of Significant Exposure to Atrazine - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference Chemical Form
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
88	Rat (Sprague-Dawley)	24 mo (F)		6.9		39.2	(CEL: increased incidence of mammary and pituitary tumors at 1 year)	Wetzel et al. 1994 97% pure

[a]The number corresponds to entries in Figure 3-1.
[b]Differences in levels of health effects and cancer effects between males and females are not indicated in Figure 3-1. Where such differences exist, only the levels of effect for the most sensitive gender are presented.
[c]An MRL of 0.01 mg/kg/day has been derived for acute-duration oral exposure (14 days or less) to atrazine based on a NOAEL of 1 mg/kg/day for decreased body weight gain in pregnant rabbits exposed to atrazine on gestational days 7-19 (Infurna et al. 1988) and an uncertainty factor of 100 (10 for extrapolation from animals to humans and 10 for human variability).

ALT = alanine aminotransferase; AP = alkaline phosphatase; Bd Wt = body weight; Cardio = cardiovascular; CEL = cancer effect level; d = day(s); DNA = deoxyribonucleic acid; Endocr - endocrine; (F) = feed; F = female; (G) = gavage; gastro = gastrointestinal; gd = gestation day; gen = generation; (GO) = gavage in oil; (GW) = gavage in water; HBDH = hydroxybutyrate dehydrogenase; Hemato = hematological; hr = hour(s); LD_{50} = lethal dose, 50% kill; LDH = lactate dehydrogenase; LH = luteinizing hormone; LOAEL = lowest-observed-adverse-effect level; M = male; Metab = metabolic; mg /kg/day = milligram per kilogram per day; mo = month(s); Musc/skel = musculoskeletal; NOAEL = no-observed-adverse-effect level; ppd = post-parturition day; RBC = red blood cell(s); Resp = respiratory; wk = week(s); x = times

Figure 3-1. Levels of Significant Exposure to Atrazine - Oral

Acute (≤14 days)

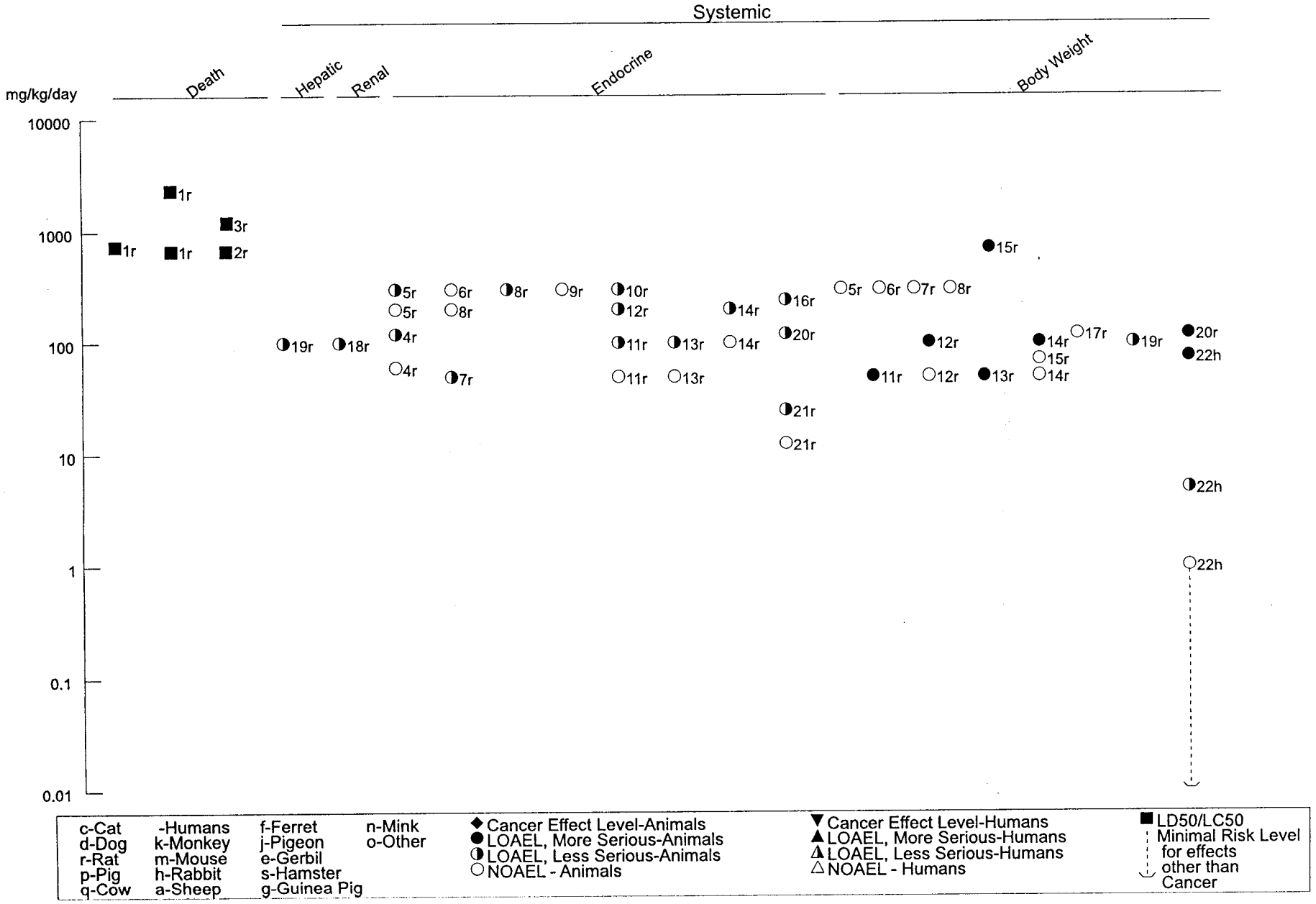


Figure 3-1. Levels of Significant Exposure to Atrazine - Oral (*continued*)

Acute (≤14 days)

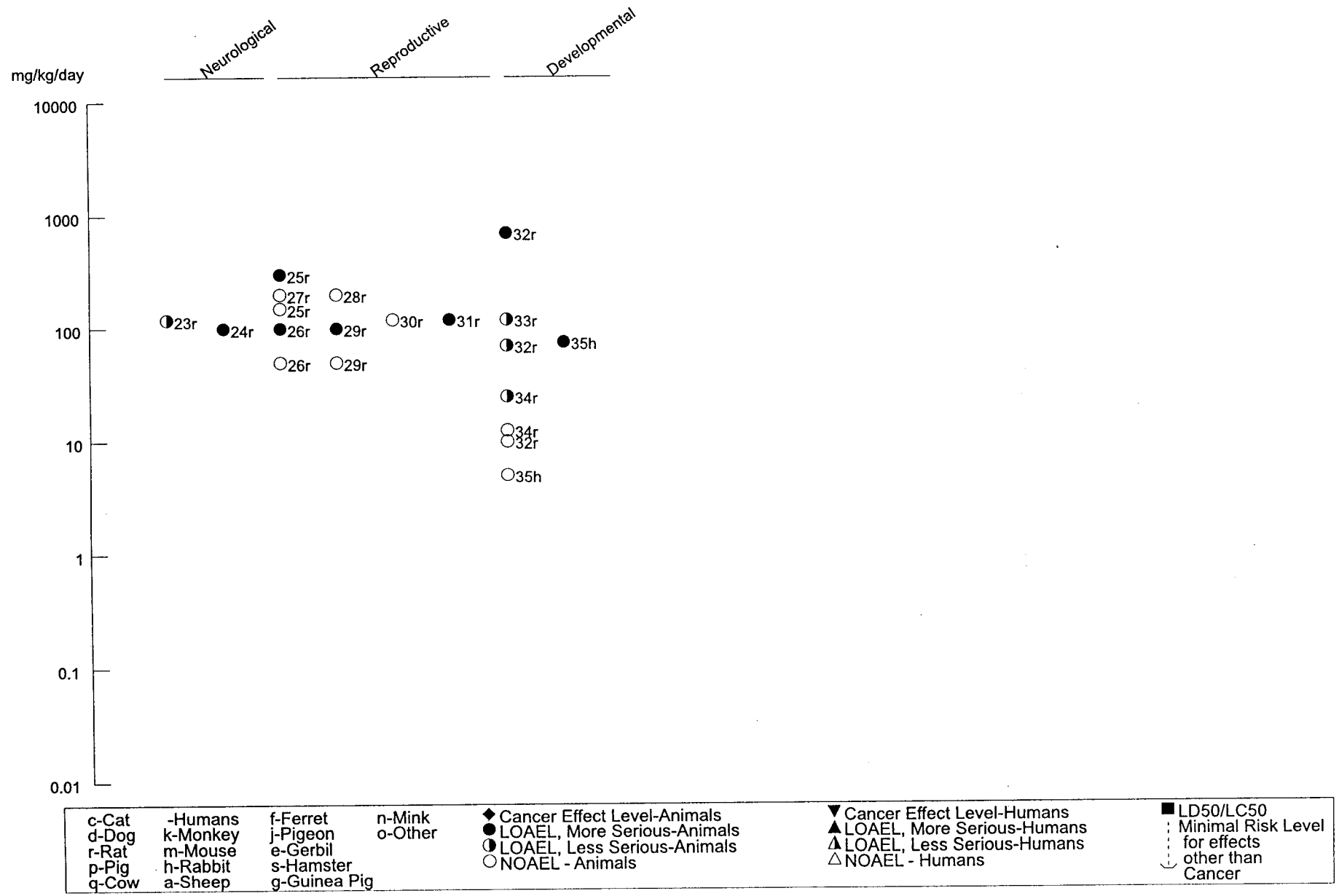


Figure 3-1. Levels of Significant Exposure to Atrazine - Oral (*continued*)

Intermediate (15-364 days)

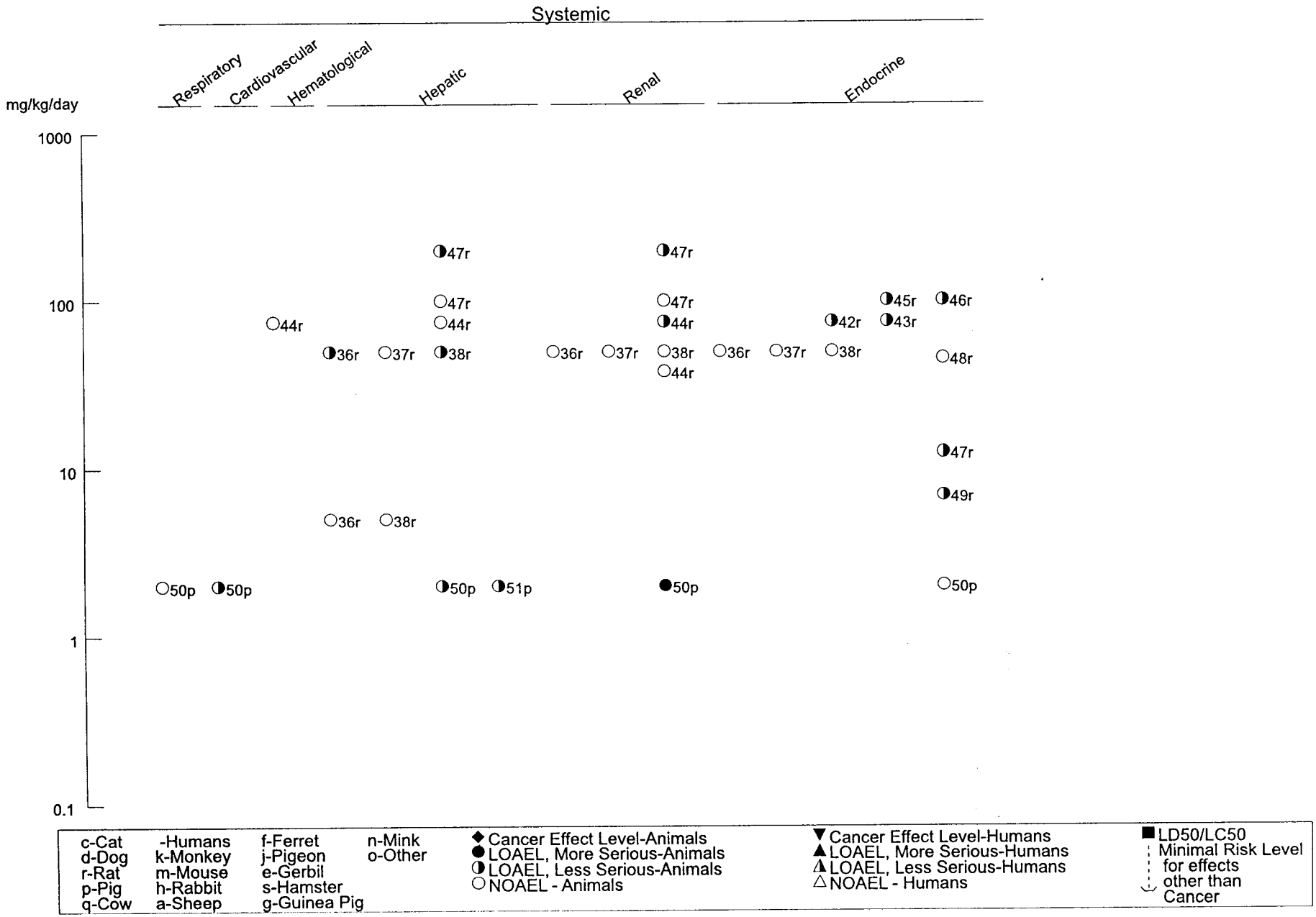


Figure 3-1. Levels of Significant Exposure to Atrazine - Oral (*continued*)

Intermediate (15-364 days)

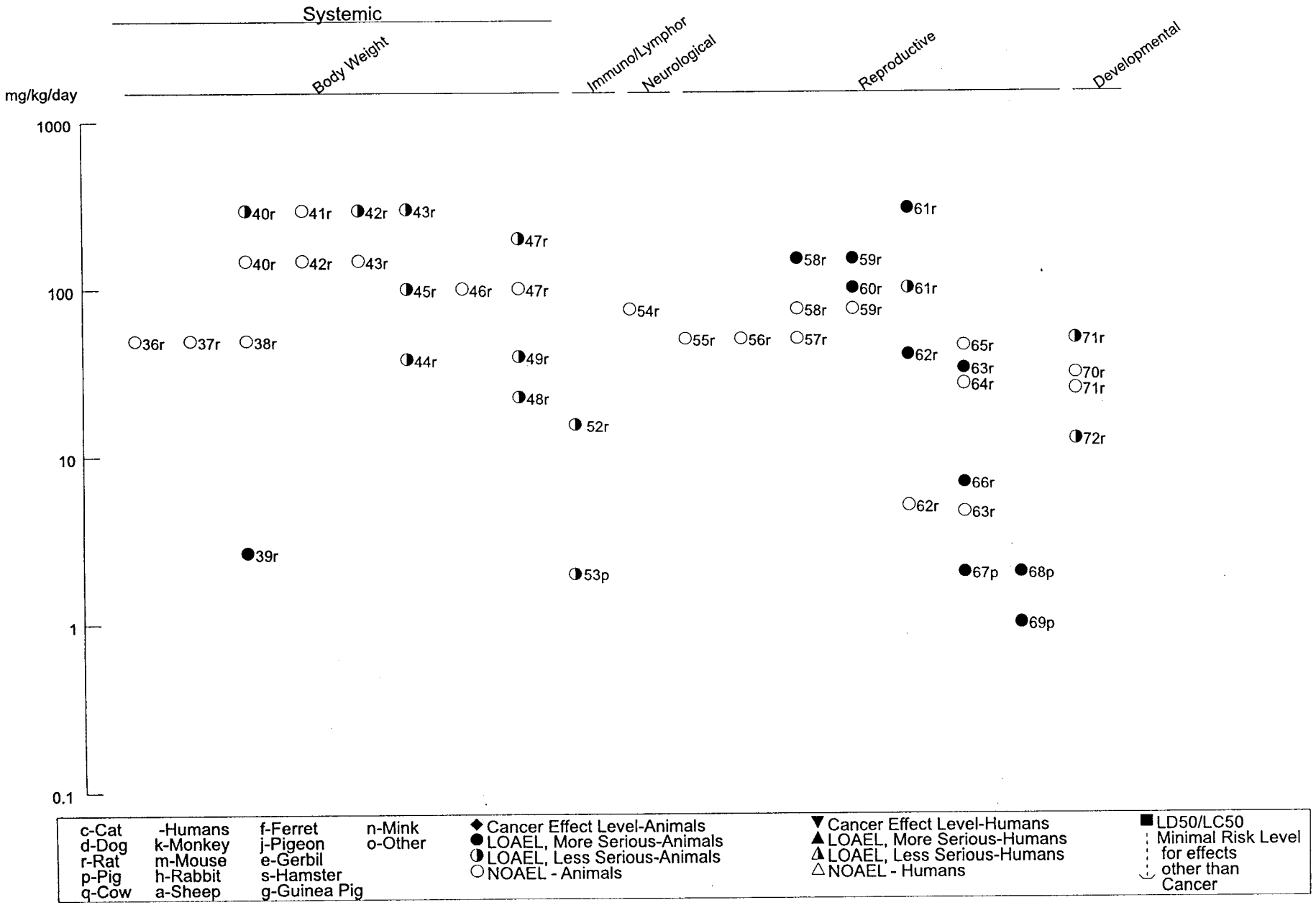


Figure 3-1. Levels of Significant Exposure to Atrazine - Oral (*continued*)

Chronic (≥365 days)

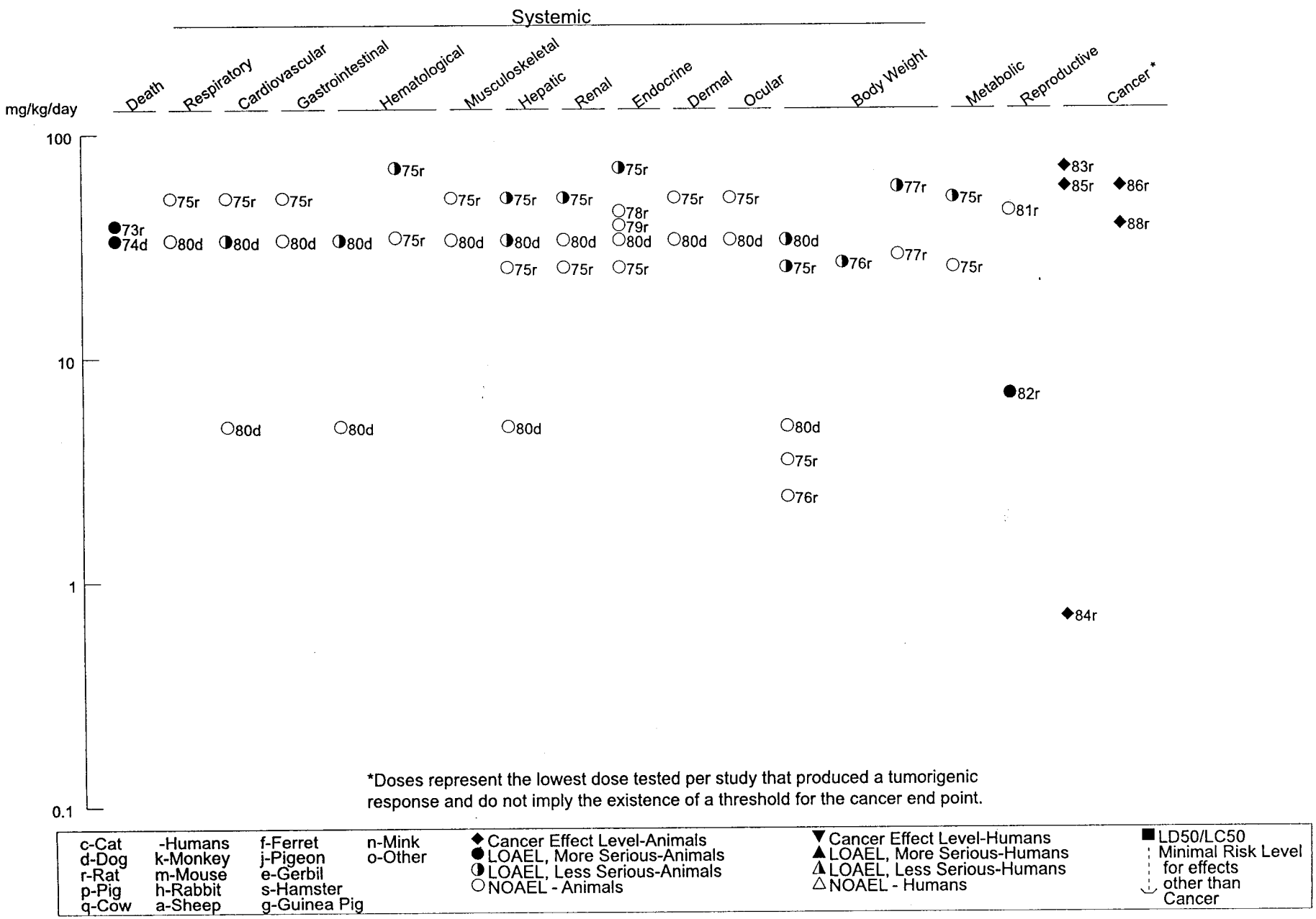


Table 3-2. Levels of Significant Exposure to Atrazine - Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)		LOAEL Serious (mg/kg)		Reference Chemical Form
ACUTE EXPOSURE								
Death								
Rat (Sherman)	single dose (dermal)					>2500	(LD_{50})	Gaines and Linder 1986 technical grade
Systemic								
Human	few hr (occup)	Dermal		NS	(acute contact dermatitis)			Schlicher and Beat 1972 NS
CHRONIC EXPOSURE								
Cancer								
Human	12mo (occup)					NS	(CEL: correlation between atrazine use and brain, testis, and prostate cancers and leukemia in Hispanic and black males)	Mills 1998 NS
Human	1-21+yr (occup)					NS	(CEL: increased risk of non-Hodgkins lymphoma)	Weisenburger 1990 NS
Human	NS (occup)							Zahm et al. 1993 NS

CEL = cancer effect level; hr = hour(s); kg = kilogram; LD_{50} = lethal dose, 50% kill; LOAEL = lowest-observed-adverse-effect level; mg = milligram; mo = month(s); NOAEL = no-observed-adverse-effect level; NS = not specified; (occup) = occupational; yr = year(s)

number of myocardial fibers in pigs exposed to 2 mg/kg/day atrazine in the feed for 19 days (, urif et al. 1999); no clinical manifestations were apparent. In contrast, no histopathological alterations were observed in male and female rats exposed to up to 52 and 70.6 mg/kg/day atrazine, respectively, in the diet for 12 months (EPA 1984a, 1987) or in sheep consuming hay sprayed with atrazine (approximately 47 mg atrazine/kg body weight/day) for 27 days (Johnson et al. 1972).

Gastrointestinal Effects. No histological alterations were observed in the gastrointestinal tracts of rats exposed to 52–70.6 mg/kg/day for 12 months (EPA 1984a, 1987a) or in sheep exposed to approximately 47 mg atrazine/kg body weight/day for 25 days (Johnson et al. 1972).

Hematological Effects. Although some animal studies have reported hematological effects, the results have been inconsistent across studies. Decreases in erythrocyte, hemoglobin, and hematocrit levels and increases in mean corpuscular hemoglobin and platelet levels were observed in female rats exposed to 70.6 mg/kg/day atrazine in the diet for 12 months (EPA 1984a, 1987a). No effects were observed in female rats exposed to 34.6 mg/kg/day or in male rats exposed to doses up to 52 mg/kg/day. Decreases in erythrocyte and hemoglobin levels and increases in platelet counts were also seen in dogs exposed to about 34 mg/kg/day atrazine for 52 weeks (EPA 1989); however, the study directors considered these changes to be secondary to decreased body weight. No alterations in erythrocyte or platelet parameters were observed in rats exposed to 75 mg/kg/day atrazine in the diet for 3 months (Dési 1983), rats exposed to 9.8–43.1 mg/kg/day atrazine in the diet for 6 months (Suschetet et al. 1974), or sheep exposed to approximately 47 mg/kg/day atrazine in the diet for 25 days (Johnson et al. 1972).

A decrease in total white blood cell counts were observed in male and female rats exposed to 43.1 and 9.8 mg/kg/day atrazine, respectively, in the diet for 6 months in male and female rats, and an increase in leukocyte levels was observed in female rats exposed to 70.6 mg/kg/day atrazine in the diet for 12 months (EPA 1984a, 1987). No alterations in leukocyte levels were observed in male rats exposed to 52 mg/kg/day for 12 months (EPA 1984a, 1987a) or in sheep consuming hay sprayed with atrazine for 25 days (Johnson et al. 1972).

Musculoskeletal Effects. No histopathological changes were noted in skeletal muscle of male or female rats exposed to up to 52 or 70.6 mg/kg/day atrazine, respectively, in the diet for 12 months (EPA 1984a, 1987a) or dogs exposed to up to 34 mg/kg/day atrazine in the diet for 52 weeks (EPA 1989).

Hepatic Effects. The available data suggest that the liver is a target of atrazine toxicity with apparent species differences in sensitivity and, therefore, in the extent of damage. Of the tested animal species, the pig appears to be the most sensitive species. Intermediate-duration exposure of pigs to 2 mg/kg/day resulted in a 350% increase in serum γ-glutamyltransferase activity and mild histopathological changes, including chronic interstitial inflammation, lymphocyte and eosinophil infiltration, and narrowing and irregular forms of bile canaliculi (Gojmerac et al. 1995). , urif et al. (1999) found similar histopathological changes in the livers of pigs exposed to 2 mg/kg/day for 19 days.

Alterations in clinical chemistry parameters and alterations in liver weight have been observed in rats, although strain differences have been observed. In Wistar rats receiving gavage doses of atrazine in gum arabic for up to 14 days (Santa Maria et al. 1987), dose-related increases in serum total lipids, alkaline phosphatase (AP) activity, and alanine aminotransferase (ALT) activity were observed at 100 mg/kg/day. Decreases in serum glucose levels and subcellular changes including proliferation and degeneration of the smooth endoplasmic reticulum, lipid accumulation, mitochondrial malformation, and alteration of bile canaliculi were observed at 200 mg/kg/day, and significantly decreased relative liver weight was observed at 400 mg/kg/day. The decreased relative liver weight may be reflective of the decreased body weight also observed in these animals. Significant decreases in serum glucose, calcium, total triglyceride, and globulin (males only) levels, and an increase in albumin/globulin ratios (males only) were observed in male and female CD rats exposed to 52 or 70.6 mg/kg/day, respectively, in the diet for 12 months; no hepatic effects were observed at 25.5 and 34.6 mg/kg/day for males and females, respectively (EPA 1984a, 1987a). Liver effects (increased relative liver weights) have also been observed in Sprague-Dawley and Donryu rats receiving gavage dose of 50 mg/kg/day, but not 5 mg/kg/day, for 28 days (Aso et al. 2000); no histological alterations were observed. No liver effects were observed in similarly exposed Fischer-344 rats (Aso et al. 2000). An increase in relative liver weight was also observed in male dogs exposed to 33.65 mg/kg/day atrazine in the diet for 52 weeks; no alterations in clinical chemistry parameters were observed. This study identified a NOAEL of 4.97 mg/kg/day. No liver effects were observed in mice receiving a single dose of up to 875 mg/kg atrazine (as the commercial product Aatrex) (Fournier et al. 1992) or sheep exposed to 47 mg/kg/day atrazine in the diet for 25 days (Johnson et al. 1972).

Renal Effects. Kidney effects have been observed in rats and pigs, but not in mice, sheep, or dogs. In male Wistar rats administered atrazine via gavage at 100 mg/kg/day or higher for 14 days, increases in urinary sodium, potassium, chloride, and protein levels, and serum lactate dehydrogenase (LDH) and γ-hydroxybutyrate dehydrogenase (HBDH) activities (considered by the study authors to be of renal, not

hepatic, origin) were observed (Santa Maria et al. 1986); this study did not identify a NOAEL. Exposure of male rats to 52 mg/kg/day atrazine in the diet for 12 months resulted in decreased kidney weight and kidney to brain weight ratios, decreased specific gravity and increased volume of urine, and increased incidence of pelvic calculi in the kidney; females exposed to 70.6 mg/kg/day had only increased relative kidney weight (EPA 1984a, 1987). In this study, no renal effects were observed at 25.5 (males) or 34.6 (females) mg/kg/day. No significant alterations in kidney weight, gross pathology, or histopathology were observed in female Sprague-Dawley, Fischer-344, and Donryu rats gavaged with up to 50 mg/kg/day for 28 days (Aso et al. 2000). The rat data suggest that males may be more sensitive to the renal toxicity of atrazine than females.

Subacute glomerulitis and degeneration and desquamation of the proximal tubules were observed in female pigs receiving 2 mg/kg/day atrazine in the diet for 19 days (, uri*f* et al. 1999). No renal effects were observed in mice administered single gavage doses of up to 875 mg/kg/day atrazine (kidney weight and gross pathology examined) (Fournier et al. 1992), in sheep receiving gavage doses of 50 mg/kg/day for 28 days (gross and histopathology examined) (Johnson et al. 1972), or in dogs administered up to 70.6 mg/kg/day atrazine in the diet for 52 weeks (gross and histopathology examined) (EPA 1989).

Endocrine Effects. Several mild to moderate endocrine effects have been observed in laboratory animals following atrazine administration, the majority of which are related to reproductive effects (see Section 3.2.2.5). The endocrine effects consisted of alterations in gland weight, histological damage in some endocrine glands, and alterations in hormone levels. A number of studies have found pituitary effects. Increased pituitary weight, hyperemia and hypertrophy, and impaired testosterone metabolism were observed in male Fischer rats administered 12 mg/kg/day atrazine by gavage for 7 days (Babic-Gojmerac et al. 1989). The levels of three testosterone metabolites (5α-androstane-3α,17β-diol, 5α-dihydrotestosterone, and androstene-3,17-dione) were decreased in the anterior pituitary suggesting impaired metabolism of testosterone. No effect on the pituitary gland were observed at 6 mg/kg/day. Increased pituitary weights were also observed in male rats gavaged with 120 mg/kg/day for 7 days, then observed for 14 days (Šimi*f* et al. 1994). Female CD rats exposed to 70.6 mg/kg/day atrazine in the diet for 12 months had an increased incidence of enlarged pituitaries (EPA 1984a, 1987a). No pituitary effects were observed in the male rats. No histological alterations were observed in the pituitary of dogs exposed to 33.80 mg/kg/day atrazine in the diet for 52 weeks (EPA 1989).

Possibly related to the effects on the pituitary are alterations in a number of pituitary-related and controlled hormones. Ovariectomized Long-Evans rats implanted with estrogen-filled silastic capsules

(which standardizes the estrogen levels and eliminates the ovary's influence on the pituitary) and administered 50 mg/kg/day atrazine or higher for 3 days had increased levels of pituitary prolactin and decreased serum prolactin levels (Cooper et al. 2000). The decrease in serum prolactin levels was also observed in similarly treated Long-Evan rats administered a single dose of 300 mg/kg/day (Cooper et al. 2000). In parallel studies, Sprague-Dawley rats treated in an identical manner and administered 300 mg/kg/day for 3 days had no increases in pituitary prolactin levels, but did have decreased serum prolactin levels (Cooper et al. 2000); a single dose of 300 mg/kg/day did not result in alterations in prolactin levels. Long-Evans and Sprague-Dawley rats treated similarly with 75–300 mg/kg/day for 21 days had increased pituitary prolactin, and the Long-Evans rats also had decreased serum luteinizing hormone and prolactin (Cooper et al. 2000). A significant increase in serum prolactin levels were observed in Sprague-Dawley rats exposed to 39.2 mg/kg/day atrazine in the diet for 9 months, but no alterations were observed after 12, 18, or 24 months of exposure (Wetzel et al. 1994). No alterations in serum prolactin levels were observed in female Fischer-344 similarly exposed to up to 45.2 mg/kg/day for 24 months (Wetzel et al. 1994). Rat dams that received $25 mg/kg/day atrazine on lactation days 1–4 had decreased prolactin release in response to pup suckling (Stoker et al. 1999).

In the studies of ovariectomized rats supplemented with estrogen (via an implanted silastic capsule), decreases in serum luteinizing hormone levels were observed at 300 mg/kg/day in Long Evans rats receiving a single dose (Cooper et al. 2000), 50 mg/kg/day in Long Evans rats receiving daily doses for 3 days (Cooper et al. 2000), 75 mg/kg/day in Long Evans rats receiving 21 doses of atrazine (Cooper et al. 2000), and 150 mg/kg/day in Sprague-Dawley rats exposed to atrazine for 21 days (Cooper et al. 2000). In ovariectomized Long Evans rats supplemented with estrogen and gonadotropin releasing hormone, a 3-day exposure to atrazine resulted in higher blood luteinizing hormone levels than in atrazine-exposed rats not receiving gonadotropin releasing hormone (Cooper et al. 2000), suggesting that atrazine disrupts neuroendocrine regulation.

The alterations in pituitary hormones result in changes in peripheral gland hormone levels. As discussed in the Reproductive Effects section, significant increases and decreases in plasma estradiol and progesterone levels have been observed in rats following acute, intermediate, or chronic duration exposure to atrazine (Cooper et al. 1996b; Cummings et al. 2000; Eldridge et al. 1994a; Wetzel et al. 1994).

Several studies have examined the adrenal glands following oral exposure to atrazine, and most studies did not find adverse effects. No alterations in adrenal weight and/or histopathology were observed in

mice receiving a single gavage dose of 875 mg/kg/day (Fournier et al. 1992), Sprague-Dawley, Fischer-344, and Donryu rats administered 50 mg/kg/day for 28 days (Aso et al. 2000), F0, F1, and F2 albino rats exposed to up to 30.9 mg/kg/day atrazine in the diet (CGC 1987), sheep exposed to up to 47 mg/kg/day atrazine for 25 days in the diet (Johnson et al. 1972), pigs that received 2 mg/kg/day in the diet for 19 days (, uri*f* et al. 1999), or dogs exposed to 33.80 mg/kg/day atrazine in the diet for 52 weeks (EPA 1989). Increases in adrenal weights were observed in female Sprague-Dawley rats and Fischer-344 rats administered by gavage 100 mg/kg/day atrazine (Eldridge et al. 1994a) and in female rats, but not males, exposed to 70.6 mg/kg/day atrazine in the diet for 12 months (EPA 1984a, 1987b).

The thyroid may also be a target of atrazine toxicity. A significant increase in relative thyroid weight was reported in Wistar rats dosed with 138.6 mg/kg/day atrazine by gavage for 3 weeks (Vos et al. 1983); because a decrease in body weight gain was also observed at this dosage, it is difficult to determine whether the increased thyroid weight was due to a direct effect of atrazine or was reflective of the decreased body weight. A decrease in serum triiodothyronine levels were observed in rats receiving gavage doses of 240 mg/kg/day atrazine for 6–12 days (Kornilovskaya et al. 1996). Histological damage to thyrocytes (decreased diameter, decreased cell height, increased), increased thyroid follicle size, and desquamation of the epithelium of the follicular cavity were also observed in these rats. No histological effects on the thyroid were reported in rats exposed to 70.6 mg/kg/day atrazine in the diet for 12 months (EPA 1984a, 1987a) and no alterations in thyroid stimulating hormone levels were observed in Long-Evans and Sprague-Dawley rats receiving gavage doses of atrazine for 1, 3, or 21 days (Cooper et al. 2000). It is not known whether the thyroid changes are direct results of atrazine toxicity or indirect results via atrazine effects on the regulation of pituitary hormones.

Dermal Effects. Information on the dermal toxicity of atrazine is limited to two studies that found no gross or histological abnormalities in the skin of male and female rats administered up to 52.0 and 70.6 mg/kg/day technical atrazine, respectively, in the diet for 12 months or in dogs that received up to about 34 mg/kg/day technical atrazine in the feed for 52 weeks (EPA 1989).

Ocular Effects. No ocular effects were noted in male and female rats administered up to 52.0 and 70.6 mg/kg/day technical atrazine, respectively, in the diet for 12 months (EPA 1984a, 1987a), or in dogs that received up to about 34 mg/kg/day technical atrazine in the feed for 52 weeks (EPA1989).

Body Weight Effects. Many rat studies involving acute, intermediate, or chronic exposure to atrazine in the diet or by gavage showed mild to severe weight loss (Cantemir et al. 1997; Cooper et al. 2000; Cummings et al. 2000; Eldridge et al. 1994a, 1999a; Infurna et al. 1988; Peruzovi*f* et al. 1995; Santa Maria et al. 1987; Šimi*f* et al. 1994; Suschetet et al. 1974; Tennant et al. 1994b; Wetzel et al. 1994). Some of these studies noted corresponding reductions in food intake (Infurna et al. 1988; Suschetet et al. 1974), and recovery following cessation of atrazine administration was noted in one study (Peruzovi*f* et al. 1995). One study in mice showed no weight loss after a single dose of up to 875 mg/kg (Fournier et al. 1992). Rabbits exposed to 75 mg/kg/day atrazine by gavage experienced severe food intake reduction and weight loss (Infurna et al. 1988). A 1-year diet study in dogs showed terminal body weights were 19 and 14% less than controls in males and females, respectively, exposed to 34 mg/kg/day atrazine and body weight gain was reduced by 17 and 14%, respectively (EPA 1989). Food intake was also decreased in these dogs by a similar amount as body weight decreased (EPA 1989).

Metabolic Effects. No studies were located regarding metabolic effects in humans or animals following oral exposure to atrazine.

3.2.2.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans after oral exposure to atrazine.

Líšková et al. (2000) performed a variety of tests to assess the immunotoxicity of atrazine in Balb/c and C57B1/10 mice. In the plaque-forming cell (PFC) assay, which tests humoral immunity by determining the integrity of three immune cells, macrophages, T cells, and B cells, administration of 100 mg/kg/day atrazine in corn oil by gavage for 10 days resulted in a 16 and 25% decrease in the number of IgM PFC per million splenic cells as compared to saline and oil controls, respectively. Other immunological effects observed in this group of mice included a decrease in spleen cellularity and a decrease in relative thymus weight. No significant alterations were observed in politeal lymph node (PLN) activation in the graft versus host and host versus graft reactions, which were used to assess the potential of atrazine to induce autoimmune disease, or the delayed-type hypersensitivity (DTH) reaction. No immunological effects were observed at 20 mg/kg/day.

Rats treated with $15.4 mg/kg/day atrazine for 3 weeks had decreased lymphocyte counts (Vos et al. 1983). Exposure to 138.6 mg/kg/day also produced increased thyroid and mesenteric lymph node

weights and decreased thymus weights (Vos et al. 1983); no increases in histological abnormalities were seen. Lymphoid depletion in the lymphoid follicles of prescapular and mesenteric lymph nodes, accompanied by infiltration of eosinophilic granulocytes, was seen in female cross-bred pigs administered 2 mg/kg/day atrazine in the feed for 19 days (, uri*f* et al. 1999). Lymphoid depletion was also seen in the lymph nodes of the white pulp of the spleen. No histopathological changes were seen in the thyroid and no clinical signs were observed (, uri*f* et al. 1999).

3.2.2.4 Neurological Effects

No studies were located regarding neurological effects in humans after oral exposure to atrazine.

A single dose of 100 mg/kg atrazine lowered the spontaneous cerebellar activity (spontaneous firing rate of Purkinje cells) of Wistar rats to 50 and 80% of control values 60 and 90 minutes, respectively, after atrazine administration (Podda et al. 1997). The evoked spike activity of Purkinje cells following stimulation of the radial nerve was almost completely abolished in atrazine-treated rats, and the amplitude of the cerebellar potentials of N2 (expression of the mossy fibers input) and CF (expression of the climbing fibers input) were reduced by 58 and 75%, respectively, 30 minutes after atrazine administration (Podda et al. 1997). Six days of oral exposure to Ceazine herbicide (used to deliver 220 mg/kg/day atrazine) resulted in decreased brain monoamine oxidase activity in Wistar rats (Bainova et al. 1979). All cerebellar activities recovered fully in 1½–2 hours. Rats treated with up to 75 mg/kg/day atrazine in the diet for 3 months showed no differences from controls in running time to the goal (food) or number of errors in behavioral maze studies (Dési 1983).

3.2.2.5 Reproductive Effects

No studies were located regarding reproductive effects in humans after oral exposure to atrazine.

Much of the research on the reproductive toxicity of atrazine has focused on the disruption of the endocrine system and its effect on estrus cyclicity. Peruzovi*f* et al. (1995) monitored estrus cyclicity in Fischer-344 rats before, during, and after atrazine exposure, which consisted of gavage administration of 120 mg/kg atrazine (purified by recrystallization) every 48 hours for a total of 6 doses. Atrazine exposure did not affect duration or frequency distribution of the individual phases of estrus. In contrast, Fischer-344 rats exposed to 120 mg/kg/day for 7 consecutive days showed a significant decrease in percent females with regular ovarian cycling, an increase in the average length of diestrus (10.5 days

compared to 2 days in controls), and an increase in the average number of days between treatment cessation and the first proestrus (6.2 days compared to 2.2 days in controls) (Šimi*ƒ* et al. 1994). Gavage dosing of 300 mg/kg/day for 3 days resulted in pseudopregnancy (defined as maintaining diestrus for 12 days or more and having elevated serum progesterone levels) in Long Evans rats; this dose also blocked the appearance of subsequent proestrus and ovulation (Cooper et al. 2000). No effect on estrus cyclicity was observed at 150 mg/kg/day. The acute data suggest that both dose and duration of exposure may be important in the atrazine-induced disruption of the estrus cycle in rats.

The intermediate-duration studies that examined atrazine-induced alterations in the estrus cycle support the findings of the acute-duration studies that the threshold of toxicity appears to be dose- and duration-related; the rat data also suggest strain differences. No statistically significant alterations in estrus cycle were observed in Sprague-Dawley, Fischer-344, or Donryu rats administered via gavage 50 mg/kg/day atrazine for 28 days (Aso et al. 2000). This study has low statistical power because of the small number of animals tested (6/group/strain). Persistent estrus was observed in one of the six Fischer-344 rats exposed to 50 mg/kg/day, one of six Donryu rats exposed to 5 mg/kg/day and one of six Donryu rats exposed to 50 mg/kg/day. At a similar exposure duration (21 days), alterations in the estrus cycle were observed in Long-Evans and Sprague-Dawley rats administered 150 or 300 mg/kg/day atrazine via gavage (Cooper et al. 1996b). The alterations consisted of a significant increase in the percentage of days in vaginal diestrus and a significant decrease in the percentage of days in vaginal estrus (not seen in Sprague-Dawley rats dosed with 150 mg/kg/day). A study by Eldridge et al. (1994a) also investigated possible strain differences among rats exposed to atrazine for <30 days. Altered estrus cyclicity was observed at 100 mg/kg/day (lowest dose tested) in Sprague-Dawley rats and 300 mg/kg/day in Fischer-344 rats administered atrazine by gavage for 14–21 days. A long-term exposure study by Wetzel et al. (1994) identified a no effect level of 45.2 mg/kg/day in Fischer-344 rats following intermediate- or chronic-duration exposure. A no effect level for estrus cycle alterations was not identified for Sprague-Dawley rats. Studies with this strain of rats showed that as the Sprague-Dawley rats aged, the effect of atrazine on the estrus cycle changed (Eldridge et al. 1999a). During the first couple of weeks of exposure to 33 mg/kg/day atrazine in the diet, an increase in diestrus was observed with no effect on the number of days in estrus. After 13–14 weeks of exposure, there was a shift in the atrazine-affected estrus cycle; the number of days in diestrus decreased and the number of days in estrus increased. This is supported by the findings of the Wetzel et al. (1994) study that significant increases in the percentage of time in estrus was seen in Sprague-Dawley rats exposed to 6.9 mg/kg/day atrazine in the diet for 1, 9, and 18 months, but not after 24 months of exposure.

The alterations in estrus cycle length most likely resulted from alterations in reproductive hormones. However, consistent alterations in reproductive hormone levels have not been observed across studies. In general, increases in plasma estradiol levels were associated with increases in percentage of days in estrus and increases in plasma progesterone levels were associated with increases in percentage of days in diestrus. A significant increase in plasma estradiol levels was observed in Sprague-Dawley rats exposed to 150 mg/kg/day atrazine via gavage for 14–23 days (Eldridge et al. 1994a). However, a decrease in plasma estradiol and an increase in plasma progesterone levels were observed at 300 mg/kg/day. The study authors suggested that this may reflect a diminished ability of rats in the 300 mg/kg/day group to develop mature ovarian follicles. An increase in estradiol levels was also observed in Sprague-Dawley rats exposed to 6.9 mg/kg/day atrazine for 3 months, but not after 1, 9, 12, 15, 18, or 24 months (Wetzel et al. 1994). In the similarly exposed Fischer-344 rats, no alterations in estradiol levels were found, and progesterone levels were not significantly altered in either strain. In the Cooper et al. (1996b) study, significant increases in plasma progesterone levels were observed in Long Evans and Sprague-Dawley rats administered 150 mg/kg/day for 21 days. Other associated effects that have been observed include decreased ovarian and/or uterine weight in rats (Eldridge et al. 1994a), and absence of corpora lutea and well-developed ovarian follicles in Long Evans rats that went into diestrus immediately after exposure initiation (Cooper et al. 1996b). Atrazine did not affect ovulation or number of ova in rats that did cycle (Cooper et al. 1996b, 2000).

Several studies have been conducted by a single group of investigators that examined the effects of atrazine ingestion in pigs (, uri*f* et al. 1999; Gojmerac et al. 1996, 1999). Pigs with observed normal estrus cycles were given 0 or 2 mg/kg body weight/day atrazine in the feed for 19 days of the estrus cycle (Gojmerac et al. 1996). The last day of treatment corresponded to day (-3) of the beginning of the next expected estrus cycle. Blood samples drawn thrice daily (at 3 hour intervals beginning at approximately 9:00 a.m.) during the first 5 days after treatment cessation showed that serum estradiol and progesterone levels were significantly altered. Estradiol levels at day (-2) of estrus is normally high and increases slightly to day (-1), then declines precipitously to day 0 and remains low during estrus. Progesterone levels during this time are normally very low from day (-2) to day 0, then gradually increase through day 2. In atrazine-treated pigs, estradiol levels were approximately 45% of normal at estrus day (-2) and remained at that level through expected estrus day 2. Progesterone levels were severely elevated (approximately 16 times normal) at estrus day (-2) and increased 3-fold to estrus day 2. These changes in hormone levels were accompanied by an absence of estrus onset. Histological examination of the ovaries showed multiple ovarian follicular cysts in various stages of development or regression, persisting corpus luteum, and cystic degeneration of secondary follicles in all treated pigs. Similar results were seen after

administration of 1 mg/kg/day atrazine (Gojmerac et al. 1999). , uri*f* et al. (1999) exposed pigs to atrazine in a similar manner to the above study and examined the thoracic and abdominal contents grossly and microscopically 9 days after treatment cessation. Again, multiple ovarian follicular cysts in various stages of development or regression, persisting corpus luteum, and cystic degeneration of secondary follicles were seen, as well as a small number of atretic follicles and normal primary and secondary follicles. The uterus was in diestrus (uterine rest) instead of in estrus.

Two studies examined the effect of atrazine on fertility. A decrease in the number of sperm positive females was seen when atrazine-exposed male and female rats were mated (Šimi*f* et al. 1994). No effect was seen when exposed males were mated with unexposed females and only a slight effect (82% sperm positive versus 100% in controls) was seen when exposed females were mated with unexposed males. No significant alterations in fertility were observed in a 2-generation rat study in which male and female Charles River albino rats were fed 26.7 mg/kg/day atrazine for at least 10 weeks prior to mating (EPA 1987b).

The highest NOAEL and all reliable LOAEL values for reproductive effects are recorded in Tables 3-1 and 3-2, and plotted in Figure 3-1.

3.2.2.6 Developmental Effects

An ecological study was conducted to assess the relationship between herbicides in the drinking water supply and intrauterine growth retardation (IUGR) (Munger et al. 1997). A survey of 856 municipal drinking water supplies in Iowa found that the Rathbun water system contained elevated levels of triazine herbicides. Several potential confounders were controlled for, including maternal smoking and socioeconomic variables. A comparison of rates of low birth weight, prematurity, and IUGR in live singleton births by women in 13 communities served by the affected water system to rates in other communities of similar size in the same Iowa counties during the period of 1984–1990 showed a greater risk of IUGR (relative risk=1.8; 95% CI=1.3, 2.7) for the Rathbun-served communities. Multiple linear regression analyses showed that levels of atrazine, metolachlor, and cyanizine were each significant predictors of community IUGR rates in the exposed communities. No definite causal relationship between any single water contaminant and risk of IUGR could be determined due to a lack of individual exposure data and the limited ability to control for confounding factors related to source of drinking water and risk of IUGR.

Developmental effects have been observed following pregestational, gestational, and lactational exposure of rat dams to atrazine. The observed effects included postimplantation losses, decreases in fetal body weight, incomplete ossification, neurodevelopmental effects, and impaired development of the reproductive system. In the offspring of Sprague-Dawley rats administered 70 mg/kg/day atrazine by gavage on gestational days 6–15, incomplete ossification of the skull, hyoid bone, teeth, forepaw metacarpals, and hindpaw distal phalanges were observed (Infurna et al. 1988). In a parallel study, pregnant rabbits administered 75 mg/kg/day atrazine on gestational days 7–19 had increased resorptions/litter and postimplantation losses/litter and decreased live fetuses/litter (Infurna et al. 1988). Decreased fetal body weights and nonossification of forepaw metacarpals and middle phalanges, hindpaw talus and middle phalanges, and patella were observed in the offspring. Severe maternal toxicity was also observed in the rabbits exposed to 75 mg/kg/day. No developmental effects were observed at 5 mg/kg/day. Holtzman rats exposed to 100, but not 50, mg/kg/day atrazine on gestational days 1–8 also had increased postimplantation losses, as well as decreased serum luteinizing hormone (LH) and progesterone (Cummings et al. 2000). Postimplantation losses were not seen at the same dose levels in Sprague-Dawley, Long-Evans, or Fischer-344 rats, although serum LH was decreased at 100–200 mg/kg/day (Cummings et al. 2000). Some differences were noted between groups of rats exposed to atrazine during the afternoon (prior to the diurnal prolactin surge) and those exposed in the early morning (prior to the nocturnal prolactin surge). No developmental effects were noted in a 2-generation study in which rats were exposed to 30.9 mg/kg/day atrazine in the diet (EPA 1987b). No alterations in the number of pups per litter or weaning weight of pups were observed in the offspring of four rats exposed to up to 112.9 mg/kg/day atrazine in the diet on gestational days 1–21 (Peters and Cook 1973).

Studies by Peruzovi*f* et al. (1995), Stoker et al. (1999, 2000), and Laws et al. (2000) examined the effect of pregestational or lactational exposure to atrazine on the development of the nervous and reproductive systems. In the Peruzovi*f* et al. (1995) study, female Fischer rats were administered via gavage 0 or 120 mg/kg purified atrazine every 48 hours for 12 days. Four weeks after the cessation of treatment, rats were mated with untreated males and allowed to carry to term and deliver pups. Litter size and pup survival were not statistically different between control and treated groups. At 70 days of age, the offspring were tested for spontaneous activity by recording ambulatory activity in 4 time blocks of 15-minutes each. At 72 days of age, avoidance response was tested by exposing each rat to a conditioning signal (a light and buzzer) followed 3 seconds later by a shock delivered through the floor of the cage that lasted up to 3 seconds. Moving to the other side of the cage within 3 seconds of the conditioning signal avoided the shock. Extinction response was tested at 73 days of age by eliminating

the shock consequence and recording the number of avoidances. Mild neurobehavioral effects were observed and differences were noted between male and female offspring. Female offspring of atrazine-treated dams had a statistically significant higher activity level than the female offspring of control dams during the first 15 minute block; no differences were seen between groups of male offspring. In the avoidance conditioning trials, male offspring of treated dams had statistically significant shorter latency times and increased number of avoidances, compared to control offspring. Conversely, female offspring of atrazine-treated dams had longer latency times and decreased number of avoidances, compared to controls, but without statistical significance. No statistical differences between treated and control groups were seen in the extinction tests (Peruzovi*f* et al. 1995).

Adult male offspring of Wistar rat dams administered up to 50 mg/kg/day atrazine on lactational days 1–4 had increased incidence and severity of inflammation of the lateral prostate, increased myeloperoxidase levels in the prostate, and increased total DNA in the prostate (Stoker et al. 1999). These effects are hypothesized to be indirect effects mediated by a lack of prolactin release in the dam in response to pup suckling; this hypothesis was supported in this study by the elimination of increased prostate inflammation in the offspring in response to co-administration of prolactin with atrazine to the dams. The level of myeloperoxidase, a lysosomal enzyme found primarily in neutrophils and macrophages, was used as an indication of the severity of inflammation. Histological examination also found increases in the incidence of focal luminal polymorphonuclear inflammation and focal interstitial mononuclear inflammation in lateral prostates at 120 days of age in the 25 and 50 mg/kg groups. Offspring of rat dams receiving atrazine on lactational days 6–9 had only statistically insignificant increases in prostate inflammation, and offspring of dams receiving atrazine on lactational days 11–14 had no increase in prostate inflammation (Stoker et al. 1999).

Male rats exposed to 50 mg/kg/day atrazine or higher on postpartum days 23–53 had decreased ventral prostate weights and delayed preputial separation, which is a marker of male puberty in the rat (Stoker et al. 2000). Dose-related increases in serum estrone and estradiol concentrations and serum T_3 were only significant in rats exposed to 200 mg/kg/day. No histopathological changes were seen in the thyroid and only mild hypospermia was seen in some high-dose rats, which may be a result of delayed puberty.

Female Wistar rats exposed to 50, but not 25, mg/kg/day atrazine from 20 to 41 days of age had delayed vaginal opening, which is a marker of female puberty in the rat (Laws et al. 2000). The age of the first 4–5-day estrus cycle after vaginal opening was also delayed; estrus cycles were normal within 3–4 weeks after cessation of atrazine exposure (Laws et al. 2000).

3.2.2.7 Cancer

An ecological study in Ontario, Canada, that examined the association of atrazine in the drinking water supply with cancer incidence rates found a positive association between atrazine levels and stomach cancer (Van Leeuwen et al. 1999). However, a negative association was noted between atrazine levels and colon cancer; it was not ascertained what may have caused this result. Data were collected and analyzed for ecodistricts; no individual data were used or provided. The average atrazine contamination level was 162.74 ng/L (range of 50–649 ng/L) and potential confounding variables, including alcohol consumption, smoking, education level, income, and occupational exposures, were considered.

In rats that had received an injection of diethylnitrosamine to initiate hepatocarcinogenesis 2 weeks before beginning atrazine administration, 53 mg/kg/day atrazine in the diet for 6 weeks resulted in no increase in hepatic glutathione S-transferase placental form, a marker for preneoplastic changes (Hasegawa and Ito 1992). Atrazine-treated rats were compared to rats that received diethylnitrosamine alone. All rats had undergone a two-thirds partial hepatectomy after 1 week of atrazine administration in order to maximize interaction between proliferation and the modification effects of atrazine.

Dietary administration of a time-weighted average of 767, but not 383, ppm atrazine (58 mg/kg/day) to male Fischer rats for 2 years resulted in a dose-related significant increase in the number of rats with malignant tumors and benign mammary gland tumors (Pintér et al. 1990); however, no significant increase was seen in any specific tumor type. Female rats receiving the same dietary level of 767 ppm (65 mg/kg/day) for 2 years had increased incidence of uterine adenocarcinoma and leukemia/lymphoma and increased number of rats with malignant tumors (Pintér et al. 1990).

In another life-long study, female Fischer rats administered up to 400 ppm atrazine in the diet (45.2 mg/kg/day) had no increased incidence of mammary or pituitary tumors (Wetzel et al. 1994). Female Sprague-Dawley rats administered 400 ppm (39.2 mg/kg/day), but not 70 ppm (6.9 mg/kg/day), atrazine in the diet had increased incidences of mammary and pituitary tumors after 1 year of treatment (Wetzel et al. 1994). There were no significant increases in malignancies in any treatment group for the entire study period (0–105 weeks), likely due to age-related increases in tumors in the controls. Mammary and pituitary tumors appeared earlier in rats treated with atrazine, apparently due to the mechanism of reproductive senescence in Sprague-Dawley rats (see Sections 3.5.2 and 3.5.3).

3.2.3 Dermal Exposure

3.2.3.1 Death

No studies regarding death in humans following dermal exposure to atrazine were located.

The acute (14-day) dermal LD_{50} in rats has been reported to be >2,500 mg/kg/day (Gaines and Linder 1986).

3.2.3.2 Systemic Effects

No studies were located regarding respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, endocrine, ocular, and body weight effects in humans and/or animals after dermal exposure to atrazine.

Dermal Effects. A 40-year-old white male farmer developed blisters on his hands and forearms one afternoon after having applied atrazine to crops in the morning using a spray rig and cleaning the plugged nozzles several times with his hands (Schlicher and Beat 1972). By 14 hours later, both hands and forearms had painful erythematous eruptions with blistering and swelling. The diagnosis was acute contact dermatitis, and treatment resulted in complete recovery. The farmer had also applied a second herbicide (Bladex=2-[4-chloro-6-ethylamino-s-triazin-2-ylamino]-2-methylpropionitrile) in the same afternoon; therefore, the exact cause of the dermatitis was not discernable.

3.2.3.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological and lymphoreticular effects in humans and/or animals after dermal exposure to atrazine.

3.2.3.4 Neurological Effects

No studies were located regarding neurological effects in humans and/or animals after dermal exposure to atrazine.

3.2.3.5 Reproductive Effects

Surveys of 1,898 farm couples living year-round on farms in Ontario, Canada, to assess reproductive effects of pesticides found that crop herbicide activity and yard herbicide activity using atrazine was associated with an increase in preterm delivery (OR=2.4, 95% CI=0.8–7.0 and OR=4.9, 95% CI=1.6–15, respectively) (Savitz et al. 1997). There was a weaker association of crop herbicide activity and yard herbicide activity using atrazine with miscarriage (OR=1.5, 95% CI=0.9–2.4 and OR=1.2, 95% CI=0.6–2.3, respectively). The risk of small for gestational age deliveries was not increased in relation to pesticide exposure and sex ratio was not altered. No specific exposure levels were available, and other pesticides were used during the period when atrazine was used; therefore, it was not possible to make a definite correlation between observed effects and atrazine exposure. It is also probable that both dermal and inhalation exposure occurred.

Another survey of 1,048 farm couples in Ontario, Canada, reporting 2,012 pregnancies was conducted during 1991–1992 to assess the influence of pesticide exposure on time to pregnancy (Curtis et al. 1999). Pesticide exposure was defined as pesticide use on the farm during the month of trying to conceive or at any time during the prior 2 months (the time in which spermatogenesis may have been affected). No route of exposure was specified; however, it is likely that both inhalation and dermal exposure occurred. A number of confounders were controlled for, including age when trying to conceive, ethnicity, smoking, caffeine consumption, alcohol use, diseases or drugs that may affect fertility, working at a hazardous job off the farm, recent full-term pregnancies, breastfeeding, method of contraception discontinued when beginning to attempt pregnancy, body mass index, and gestational age at pregnancy diagnosis. Atrazine was not associated with any decrease in fecundity; in fact, atrazine was one of the eight pesticide categories associated with a 10% increase in fecundity. The study authors speculated that this association may have been due to uncontrolled confounding factors or chance.

No studies were located regarding reproductive effects in animals after dermal exposure to atrazine.

3.2.3.6 Developmental Effects

No studies were located regarding developmental effects in humans and/or animals after dermal exposure to atrazine.

3.2.3.7 Cancer

An ecological study that assessed the correlation of the amount of atrazine used in California counties to the incidence rates of each of several cancer types (non-Hodgkin's lymphoma, leukemia, soft-tissue sarcoma, brain cancer, prostate cancer, and testicular cancer) found a correlation between atrazine use and brain and testis cancers and leukemia in Hispanic males, and prostate cancer in black males (Mills 1998). However, no individual exposure data were available, no latency period was allowed for, and no allowance was made for possible changes in usage of and nonactive ingredients in pesticides over time. A population-based case-control study of 201 white men in 66 counties in eastern Nebraska who had histologically confirmed non-Hodgkin's lymphoma initially found an association between atrazine use and an elevated risk for non-Hodgkin's lymphoma (OR=3.3, 95% CI=0.5, 22.1), and that risk increased with duration (OR=0.9, 0.8, 2.0, and 2.0 for use 1–5, 6–15, 16–20, and 21+ years, respectively) (Weisenburger 1990). However, further evaluation of the study results and adjustment for use of 2,4-D and organophosphates eliminated the risks associated with long-term atrazine use (ORs fell below unity) (Zahm et al. 1993). Zahm et al. (1993) also found only a very weak association (OR=1.2, 95% CI=0.9–1.7) between atrazine use and non-Hodgkin's lymphoma in an analysis of three previous studies combined (one in Nebraska, one in Kansas, and one in Iow-Minnesota) after adjustment for 2,4-D and organophosphate use was made. Data from 173 white men with histologically diagnosed multiple myeloma and 650 controls were analyzed to determine the association between general farming activities and use on the farm of 24 animal insecticides, 34 crop insecticides, 38 herbicides, and 16 fungicides and the risk of multiple myeloma (Brown et al. 1993). Risks for multiple myeloma were not increased significantly for farmers who personally handled, mixed, or applied any specific insecticide or herbicide, including atrazine (OR for atrazine 0.8, 95% CI=0.4–1.6).

A Cancer Assessment Review Committee (CARC) meeting by the Office of Prevention, Pesticides and Toxic Substances has recently evaluated atrazine and classified atrazine as "not likely to be carcinogenic to humans" (EPA 2000a). IARC has classified atrazine in Group 3 (not classifiable as to its carcinogenicity to humans) based on inadequate evidence in humans and sufficient evidence in experimental animals (IARC 1999).

No studies were located regarding cancer in animals after dermal exposure to atrazine.

3.2.4 Other Routes of Exposure

Hematological Effects. Mice injected intraperitoneally with a single dose of 58.65 mg/kg atrazine showed changes in some hematological parameters (Mencoboni et al. 1992). Transient, but precipitous, decreases were seen in peripheral blood reticulocytes, bone marrow morphologically recognizable precursors, granulocyte-macrophage committed progenitors, and pluripotent stem cells. Peripheral blood leukocytes were not altered.

Immunological Effects. Altered immunological parameters have been observed in rats exposed to atrazine intratracheally (Hurbankova et al. 1996). Adult male Fischer-344 rats were administered a single dose of 30 mg/kg atrazine intratracheally, and at 1 week and 3 months postexposure, the tracheas were washed with saline and the wash was analyzed for the following: number of alveolar macrophages per mL; alveolar macrophage:granulocyte ratio; phagocytic activity of alveolar macrophages; viability of alveolar macrophages; size of alveolar macrophages; lactate dehydrogenase (LDH) levels; and acid phosphatase (AcP) levels. Peripheral blood was drawn and analyzed for the following: number of leukocytes/mm^3; phagocytic activity of granulocytes and monocytes; differential cell counts (granulocytes, monocytes, lymphocytes); LDH levels in serum; and AcP levels in serum. One week after exposure, the statistically significant changes were: increased number of aveolar macrophages; decreased percent of active phagocytes; increased LDH in bronchoalveolar lavage; decreased % monocytes in blood; increased LDH in serum; and increased AcP in serum. Three months after exposure, the percent of active phagocytes and AcP in serum were still statistically significantly altered.

Neurological Effects. Sprague-Dawley rats injected intraperitoneally with 0, 85, or 170 mg/kg atrazine twice a week for 30 days showed some transient neurological effects (Castano et al. 1982). No alterations were seen electron microscopically in the cervical or thoracic ganglia, spinal cord, or sciatic nerve of rats killed immediately after the end of the treatment period. However, morpho-quantitative analysis revealed decreased areas for myelinated and unmyelinated axons in the 170 mg/kg group; statistical significance was reached only for unmyelinated axons. Recovery was seen after 30 days of nontreatment. Morpho-quantitative analysis involved computer analysis of electron micrographs of the sciatic nerve for cross-sectional area of myelinated and unmyelinated fibers and for thickness of myelin sheaths.

Reproductive Effects. In adult male Fischer rats administered 0, 60, or 120 mg/kg/day atrazine intraperitoneally twice a week a period of 60 days, relative weights of the pituitary and ventral prostate were significantly decreased in both treatment groups (Kniewald et al. 2000). Testicular sperm numbers were increased in both treatment groups, and a dose-related decrease in epididymal sperm number was seen; testicular sperm numbers in controls decreased during the study, indicating normal sperm migration to the epididymis. Epididymal sperm motility was also decreased in both treatment groups by about 50% (motility in controls was about 50% and in treated groups was 21–25%). The activity of alpha-glucosidase in the epididymis was decreased in both treatment groups. Histological examination revealed decreased spermatogenesis and cell disorganization. Electron microscopy showed interstitial cells with acidophilic, differently vacuolated cytoplasm and smooth nuclei with visible nucleoli, lower cell density, and a decrease in the unit number of cells; collagen fibers were reduced and dispersed in the interstitial space; Leydig cells were small and misshapen with cytoplasms filled with lysosomes and vacuoles and the nucleus was invaginated; the morphology of the rough and smooth endoplasmic reticulum in Leydig cells was altered; and degenerative changes were seen in Sertoli cells.

Developmental Effects. Peters and Cook (1973) conducted a set of studies examining the subcutaneous administration of high doses of atrazine to pregnant rats to determine the effects on live pups/litter and resorption sites. In rat dams exposed on gestational days 3, 6, and 9, postimplantation losses were increased at $800 mg/kg/treatment day, but not at 200 mg/kg/treatment day. In dams exposed for only 1 day (gestational day 3, 6, or 9), no dose-related increases in postimplantation losses were observed (Peters and Cook 1973). Although pups/litter were decreased in the 1,000 mg/kg group exposed on gestational day 6, there was no effect in the 2,000 mg/kg group exposed similarly.

3.3 GENOTOXICITY

Numerous *in vivo* and *in vitro* studies have assessed the genotoxic potential of atrazine, and the results of these studies are presented in Tables 3-3 and 3-4, respectively.

Several studies have examined the *in vivo* genotoxicity of atrazine in rats, mice, and *Drosophila*; no *in vivo* human genotoxicity studies were located. An increased occurrence of DNA strand breaks were observed in the stomach, liver, and kidneys, but not in the lungs, of rats that received a single dose of 875 mg/kg or 15 daily doses of 350 mg/kg atrazine (Pino et al. 1988). An increased occurrence of micronucleus formation was observed in the bone marrow of female NMRI mice receiving a single doses of 1,400 mg/kg/day, but not in bone marrow cells from male mice dosed with 1,750 mg/kg (Gebel et al.

Table 3-3. Genotoxicity of Atrazine *In Vivo*

Species (test system)	End point	Results	Reference
Mammalian cells:			
Rat stomach, liver, kidney	DNA strand breaks	+	Pino et al. 1988
Rat lung	DNA strand breaks	–	Pino et al. 1988
Mouse bone marrow, female	Micronucleus formation	+	Gebel et al. 1997
Mouse bone marrow, male	Micronucleus formation	–	Gebel et al. 1997
Mouse bone marrow	Chromosomal aberrations	–	Meisner et al. 1992
Nonmammalian cells:			
Drosophila melanogaster	Somatic mutation	+	Torres et al. 1992
D. melanogaster	Somatic mutation	+	Tripathy et al. 1993
D. melanogaster	Dominant lethal mutation	+	Murnik and Nash 1977
D. melanogaster	Aneuploidy	+	Murnik and Nash 1977

– = negative result; + = positive result; DNA = deoxyribonucleic acid

Table 3-4. Genotoxicity of Atrazine *In Vitro*

Species (test system)	End point	With activation	Without activation	Reference
Prokaryotic organisms:				
Salmonella typhimurium	Forward mutation	–	–	Adler 1980
S. typhimurium	Reverse mutation	–	–	Kappas 1988
S. typhimurium	Reverse mutation	+	No data	Means et al. 1988
S. typhimurium	Reverse mutation	–	–	Adler 1980; Morichetti et al. 1992; Ruiz and Marzin 1997; Zeiger et al. 1988
S. typhimurium	Reverse mutation	No data	–	Andersen et al. 1972; Butler and Hoagland 1989; Seiler 1973
Esherichia coli PQ37	SOS repair	–	–	Ruiz and Marzin 1997
E. coli	Forward mutation	–	–	Adler 1980
Bacteriophage T4	Forward mutation	No data	–	Andersen et al. 1972
Bacteriophage	Reverse mutation	No data	–	Andersen et al. 1972
Eukaryotic organisms:				
Saccharomyces cerevisiae	Mitotic recombination	No data	–	Emnova et al. 1987
S. cerevisiae	Gene conversion	+	–	Plewa and Gentile 1976
S. cerevisiae	Gene conversion	–	–	Adler 1980
S. cerevisiae	Gene conversion, stationary phase	+	–	Morichetti et al. 1992
S. cerevisiae	Gene conversion, logarithmic phase	+	–	Morichetti et al. 1992
S. cerevisiae	Reverse mutation, stationary phase	No data	–	Morichetti et al. 1992
S. cerevisiae	Reverse mutation, logarithmic phase	No data	+	Morichetti et al. 1992
S. cerevisiae	Forward mutation	No data	+	Emnova et al. 1987
Aspergillus nidulans	Gene conversion	No data	–	de Bertoldi et al. 1980
A. nidulans	Mitotic recombination	+	–	Adler 1980
A. nidulans	Mitotic recombination	–	–	Kappas 1988
A. nidulans	Forward mutation	+	–	Benigni et al. 1979
A. nidulans	Aneuploidy	+	–	Benigni et al. 1979
Schizosaccharomyces pombe	Reverse mutation	+	–	Mathias et al. 1989
Tradescantia paludosa	Micronucleus formation	+	–	Mohammed and Ma 1999

Table 3-4. Genotoxicity of Atrazine *In Vitro (continued)*

Species (test system)	End point	With activation	Without activation	Reference
Mammalian cells:				
Human lymphocytes	DNA damage	–	+	Ribas et al. 1995
Human lymphocytes	Sister chromatid exchange	–	–	Dunkelberg et al. 1994
Human lymphocytes	Chromosomal aberrations	No data	+	Meisner et al. 1992, 1993

– = negative result; + = positive result; DNA = deoxyribonucleic acid

1997). Tests for somatic mutation (Torres et al. 1992; Tripathy et al. 1993), dominant lethal mutations (Murnick and Nash 1977) and aneuploidy (Murnick and Nash 1977) in *Drosophila melanogaster* have been positive.

A number of *in vitro* studies have examined the genotoxicity of atrazine in bacterial, yeast, and human lymphocyte assays. In general, atrazine did not increase the formation of forward mutations (Adler 1980) or reverse mutations (Adler 1980; Andersen et al. 1972; Butler and Hoagland 1989; Morichetti et al. 1992; Ruiz and Marzin 1997; Seiler 1973; Zeiger et al. 1988) in *Salmonella typhimurium* with or without metabolic activation. Studies in *Escherichia coli* have been negative for SOS repair (Ruiz and Marzin 1997), and forward mutations (Adler 1980); the occurrence of forward or reverse mutations were also not increased in bacteriophages (Andersen et al. 1972). In contrast to the results found in prokaryotic organisms, most assays in eukaryotic organisms showed evidence of genotoxicity. Increases in the occurrence of gene conversion (Morichetti et al. 1992; Plewa and Gentile 1976), reverse mutations (Morichetti et al. 1992), and forward mutations (Emnova et al. 1987) were observed in *Saccharomyces cerevisiae*. In *Aspergillus nidulans*, increases in the occurrence of mitotic recombination (Adler 1980), forward mutation (Benigni et al. 1979), and aneuploidy (Benigni et al. 1979) were observed. Reverse mutations in *Schizosaccharomyces pombe* (Mathias et al. 1989) and micronucleus formation in *Tradescantia paludosa* (Mohammed and Ma 1999) have also been reported. In human lymphocytes, an increase in DNA damage (Ribas et al. 1995) and chromosomal aberrations (Meisner et al. 1992, 1993) were observed; the occurrence of sister chromatid exchange was not altered (Dunkelberg et al. 1994).

3.4 TOXICOKINETICS

3.4.1 Absorption

3.4.1.1 Inhalation Exposure

No studies were located that measured absorption or monitored metabolites in excreta of humans or animals exposed to atrazine only via the respiratory route. The only available inhalation toxicity studies involved exposure to very large atrazine particles (30–70 μm) (Catenacci et al. 1990, 1993), which made it unlikely that any significant amount of atrazine reached the lungs.

3.4.1.2 Oral Exposure

Absorption of atrazine in humans following oral exposure was indicated in a single case report of a 38-year-old man who died of progressive organ failure and shock 3 days after ingesting 500 mL of a weedkiller that contained 100 g atrazine, 25 g of aminotriazole, 25 g of ethylene glycol, and 0.15 g of formaldehyde (Pommery et al. 1993). At autopsy, atrazine was detected in the kidney, small intestine, lung, liver, pancreas, muscle, heart, and plasma.

In rats gavaged with a single dose of 30 mg/kg [^{14}C]-atrazine in aqueous solution, radioactivity levels in plasma peaked 8–10 hours postdosing (Timchalk et al. 1990). The absorption of radioactivity (K_a) was described as a first-order process and was used to calculate an absorption half-life of 2.6 hours. Fifty-seven percent of the administered radioactivity was excreted in the urine within 24 hours, and only 14% in the feces, indicating a high degree of absorption. Likewise, Meli et al. (1992) recovered about 37%, as detected by gas chromatography-mass spectrometry (GC-MS), of an administered oral dose of 50 mg/kg nonradiolabeled atrazine in the urine of rats.

3.4.1.3 Dermal Exposure

Data regarding dermal exposure to atrazine in humans indicates that limited absorption occurs. Buchholz et al. (1999) applied dermal patches containing ring-radiolabeled atrazine mixed with the commercial atrazine product Aatrex to the forearms of 10 healthy male subjects for 24 hours. Unabsorbed radioactivity and the radioactivity excreted in urine and feces were measured for the 7-day period including and following the application. Six to 10 percent of the applied doses of 0.167 or 1.98 mg of atrazine was absorbed, as indicated by the unabsorbed radioactivity, but only 0.3–5.1% of the applied dose was recovered in the urine and feces. An *in vitro* study using human skin samples exposed to [^{14}C]-atrazine found that approximately 16.4% was absorbed in a 24-hour period, and that most of the absorbed atrazine (12% of the applied dose) remained in the skin (Ademola et al. 1993). Less than 5% progressed through the skin and into receptor fluid. Dermal absorption of atrazine in humans has also been indicated by occupational studies that found atrazine and its metabolites in the urine of workers exposed primarily via dermal contact (Catanacci et al. 1990, 1993).

A single study in rats compared the dermal absorption of [^{14}C]-atrazine in young and adult rats (Hall et al. 1988) by measuring the fractional skin penetration (radioactivity in the body, skin, and excreta divided by the total radioactivity recovered in the body, skin, excreta, and unabsorbed atrazine on the application

blister). The fractional skin penetration values indicated slightly higher absorption in young rats (3.2–9.6%) than in adult rats (2.8–7.7%), and decreased percent absorption with increasing atrazine dose. It is unclear what caused the difference in absorption between young and adult rats; skin thickness was almost identical in the two groups and, therefore, was not a factor. No data are available on the transport mechanism of atrazine in skin. Dermal absorption may be limited by saturation of the transport mechanism or by physical/chemical restrictions and interactions; this hypothesis is supported by an *in vitro* study showing a percentage decrease in metabolite formation with increasing atrazine dose to human skin samples (Ademola et al. 1993).

3.4.1.4 Other Routes of Exposure

No studies were located regarding absorption of atrazine after other routes of exposure in humans or animals.

3.4.2 Distribution

3.4.2.1 Inhalation Exposure

No studies were located regarding distribution of atrazine after inhalation exposure in humans or animals.

3.4.2.2 Oral Exposure

Data on distribution of atrazine in humans after oral exposure was limited to a single case report of a 38-year-old man who died of progressive organ failure and shock 3 days after ingesting 500 mL of a weedkiller that contained 100 g atrazine, 25 g of aminotriazole, 25 g of ethylene glycol, and 0.15 g of formaldehyde (Pommery et al. 1993). At autopsy, atrazine was detected in the kidney, small intestine, lung, liver, pancreas, muscle, heart, and plasma.

In male Fischer rats that received a single dose of 30 mg/kg [^{14}C]-atrazine by gavage, plasma levels of radioactivity peaked at 8–10 hours postdosing and the rate of clearance was apparently first-order with a half-life of 10.8–11.2 hours (Timchalk et al. 1990). Radioactivity was also determined for the whole skin and for the rest of the carcass and found to be 1.5 and 4%, respectively, of the administered dose.

In rats administered about 1.5 or 17.7 mg/kg [^{14}C]-atrazine by gavage, the majority of the radioactivity was recovered in the urine (65.5%) and feces (20.3%) over the course of 8 days (Bakke et al. 1972). The whole carcass contained 15.8% of the radioactivity 3 days after exposure, and radioactivity was detected in liver, brain, heart, lung, kidney, digestive tract, omental fat, and skeletal muscle on days 2, 4, and 8, and the levels decreased over time.

3.4.2.3 Dermal Exposure

No studies were located regarding distribution of atrazine after dermal exposure in humans or animals.

3.4.2.4 Other Routes of Exposure

No studies were located regarding distribution of atrazine in humans or animals exposed by routes other than oral, inhalation, or dermal.

3.4.3 Metabolism

Atrazine is extensively and rapidly metabolized as indicated by plasma levels of atrazine and the relative amounts of metabolites and parent compound in the urine within 8–24 hours after exposure. Plasma levels of ^{14}C from radiolabeled atrazine have been shown to peak at 8–10 hours postexposure in rats, and the elimination half-life has been calculated to be 10.8–11.2 hours (Timchalk et al. 1990). In urine, unchanged atrazine has been detected, but comprised <2% of all atrazine-related compounds after dermal exposure in humans (Buchholz et al. 1999; Catenacci et al. 1993) or oral exposure in rats (Meli et al. 1992). In humans, 50% of all urinary atrazine metabolites were excreted within 8 hours and 100% within 24 hours (Catenacci et al. 1993). In rats, approximately 57% of the radioactivity from administered [^{14}C]-atrazine was excreted in the urine within 24 hours (Timchalk et al. 1990), and urinary atrazine metabolites decreased to 1/30 or less of the 24-hour level by 48 hours postexposure (Meli et al. 1992).

Atrazine is primarily metabolized in humans via dealkylation, probably followed by glutathione conjugation and conversion to mercapturic acids. In humans exposed to [^{14}C]-atrazine dermally (via a patch on the forearm) for 24 hours, atrazine mercapturate was positively identified and a variety of other metabolites (deethylatrazine, didealkylatrazine and didealkylatrazine mercapturate, deethylatrazine mercapturate, and deisopropylatrazine) were tentatively identified (Buchholz et al. 1999). Metabolites found in the urine of male workers in an atrazine production plant were didealkylated atrazine (80%),

deisopropylatrazine (10%), deethylatrazine (8%), and unmodified atrazine (1–2%) (Catenacci et al. 1993). Atrazine has also been shown to be metabolized to the mono- and di-dealkylated derivatives in human skin samples *in vitro* (Ademola et al. 1993). These human data are supported by *in vivo* animal data showing the same mono- and di-dealkylated and mercapturic acid atrazine metabolites in rat urine (Bakke et al. 1972; Meli et al. 1992; Timchalk et al. 1990) and tissues (Gojmerac and Kniewald 1989) and in chicken excreta (Foster and Khan 1976). The presence of mercapturic acid metabolites in human and rat urine indicates that phase II metabolism of atrazine probably proceeds via glutathione conjugation and conversion to mercapturic acids in the kidneys before excretion.

In vitro studies using microsomal preparations from liver and other tissues of humans and animals have indicated that dealkylation of atrazine is mediated by cytochrome P-450 enzymes (CYPs) (Adams et al. 1990; Ademola et al. 1993; Croce et al. 1996; Hanioka et al. 1998a, 1999; Lang et al. 1996, 1997; Meli et al. 1992; Venkatesh et al. 1992). Ademola et al. (1993) observed a lack of atrazine metabolism in human skin microsomal preparations in the absence of NADPH and a 70% reduction in the rate of metabolite formation when the CYP inhibitor, SKF 525-A, was added to the mixture. A similar requirement of NADPH for atrazine metabolism was noted in liver microsomal preparations of all species tested. Adams et al. (1990) determined that NADH, and therefore cytochrome b_5, were not necessary and did not contribute to atrazine metabolism in microsomal preparations. Lang et al. (1997) performed a series of experiments to determine the CYP(s) responsible for atrazine metabolism in human liver microsomes. Inclusion of seven inhibitors of specific CYPs in separate microsomal incubations showed that only α-naphthoflavone and furafylline, two CYP1A2 inhibitors, inhibited the production of dealkylation products. Additionally, when cDNA-expressed CYPs (1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, and 3A4) were used in incubations similar to microsomal preparations, CYP1A2, and to a lesser extent 2C19 and 1A1, produced deisopropyl- and deethylatrazine (Lang et al. 1997). These data implicate CYP1A2 as the primary enzyme involved in phase I metabolism of atrazine in humans. In contrast, SKF 525-A, benzphetamine, and testosterone (all CYP2B1 and 2C11 inhibitors) inhibited atrazine metabolism in rat microsome incubations, while no inhibition was seen with theophylline and nicotine (CYP1A2 inhibitors) (Hanioka et al. 1998a). Thus, some species-specificity regarding phase I metabolism of atrazine is evident.

Adams et al. (1990) examined the phase II portion of atrazine metabolism *in vitro* by incubating Sprague-Dawley and Fischer rat hepatic supernatant fractions (S-10) with [^{14}C]-atrazine and glutathione in a reaction mixture for 2 hours at 37 EC. Analysis of the products showed that phase I reactions proceeded more rapidly, with only 4% of the labeled metabolites recovered in the phase II portion. It was also noted

that, in this *in vitro* system, most of the conjugated products were parent compound and not dealkylated metabolites. Phase II metabolism of atrazine was further demonstrated in another *in vitro* study that examined the activity of glutathione S-transferase (GST), the enzyme responsible for glutathione conjugation of atrazine, in cytosolic supernatants from Sprague-Dawley rats and Swiss-derived CD-1, C57BL/6, DBA/2, and Swiss-Webster mice (Egaas et al. 1995). Atrazine conjugates were detected in rats and in all strains of mice tested. These data support phase II metabolism of atrazine through glutathione conjugation and mercapturic acid formation.

While there are many similarities between and within species in phase I and phase II metabolism of atrazine, differences have also been noted. The products of phase I metabolism of atrazine have been shown to be qualitatively the same, but the rates of formation of the products and the ratio of the products was frequently different between species. Lang et al. (1996) found that the rate of formation of primary dealkylation products in human microsomes was up to 20-fold less than in rat microsomes, and the ratio of products was also different between humans, rats, and pigs. Hanioka et al. (1999) and Adams et al. (1990) found up to a 10-fold difference in rate of primary metabolite formation between rats, mice, guinea pigs, rabbits, pigs, sheep, goats, and chickens. There is also evidence of inter- and intra-species differences in phase II metabolism of atrazine. GST activity in rat liver cytosolic supernatants was much lower toward atrazine than in mice liver supernatants (about 6–37% of mouse activity) (Egaas et al. 1995). GST activity in female mouse supernatants was approximately 12–32% of that in males of the same strain, and remained constant between adolescence and sexual maturity (Egaas et al. 1995). In male mice, GST activity was much higher in the livers of sexually mature mice in all mouse strains tested except the C57BL/6, and was twice the level seen in adolescent mice of the CD-1 and Swiss-Webster strains.

3.4.4 Elimination and Excretion

Specific data on elimination and excretion of atrazine by any route were limited. However, the primary route of excretion appears to be in urine, as indicated by the detection of urinary atrazine and its metabolites in a number of species exposed via oral and dermal routes (Bakke et al. 1972; Buchholz et al. 1999; Catenacci et al. 1990, 1993; Meli et al. 1992; Timchalk et al. 1990). Fecal excretion was a minor route (Buchholz et al. 1999; Timchalk et al. 1990). No data were located regarding enterohepatic circulation and biliary secretion or excretion of atrazine in breast milk.

3.4.4.1 Inhalation Exposure

No studies were located regarding the elimination and excretion of atrazine following inhalation exposure in humans or animals.

3.4.4.2 Oral Exposure

No studies were located regarding the elimination and excretion of atrazine following oral exposure in humans.

Male Fischer-344 rats administered 30 mg/kg of [^{14}C]-atrazine by gavage eliminated 93% of the administered radioactivity within 72 hours (Timchalk et al. 1990). The primary route of excretion was in urine (67%); 36 and 21% of the administered radioactivity was eliminated in the 0–12- and 12–24-hour postexposure intervals, respectively. Fecal excretion accounted for 18% of the administered radioactivity. The elimination of atrazine from plasma followed first-order kinetics and the elimination half-life was calculated to be 10.8 hours (Timchalk et al. 1990). In rats that received a single dose of 50 mg/kg atrazine by gavage, atrazine and its metabolites were present in urine 24 hours postexposure and at 48 hours at a fraction of the 24-hour level (Meli et al. 1992).

3.4.4.3 Dermal Exposure

Doses of 0.167 mg (6.45 µCi) or 1.98 mg (24.7 µCi) of [^{14}C]-atrazine was applied to 25 cm^2 of the forearm of healthy males for 24 hours (Buchholz et al. 1999). Urinary excretion varied widely, accounting for 72, 30, and 3.5% of radioactivity absorbed by one low-dose and two high-dose individuals, respectively. Fecal excretion also varied, accounting for 11.5, 4.2, and 0%, respectively, of the absorbed radioactivity.

Urine was collected from six male workers at an atrazine production plant for 24 hours during and after an 8-hour workshift and analyzed for atrazine and atrazine metabolites (Catenacci et al. 1993). Fifty percent of the atrazine-related compound detected in the urine during the 24-hour period were excreted in the first 8 hours. A related study that measured only atrazine found that urinary levels were highest during and immediately after workshifts; levels 12 hours after the end of the workshift were one-tenth of the levels during the workshift (Catenacci et al. 1990).

3.4.4.4 Other Routes of Exposure

Lu et al. (1997b) examined the atrazine levels in the saliva of rats continuously infused with atrazine through a cannula in the femoral vein. Salivary rates were stimulated and controlled with intravenous injections of pilocarpine. Salivary and plasma levels of atrazine were simultaneously monitored over 200–300 minutes. Salivary atrazine levels remained relatively constant over a range of salivary flow rates, and the salivary/plasma concentration ratio remained fairly constant with changing salivary flow rates and plasma atrazine concentrations. The salivary atrazine concentration was found to be highly correlated with the plasma atrazine concentration.

3.4.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models. PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen

1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites) based on the results of studies where doses were higher or were administered in different species. Figure 3-2 shows a conceptualized representation of a PBPK model.

No PBPK models for atrazine were identified in the literature.

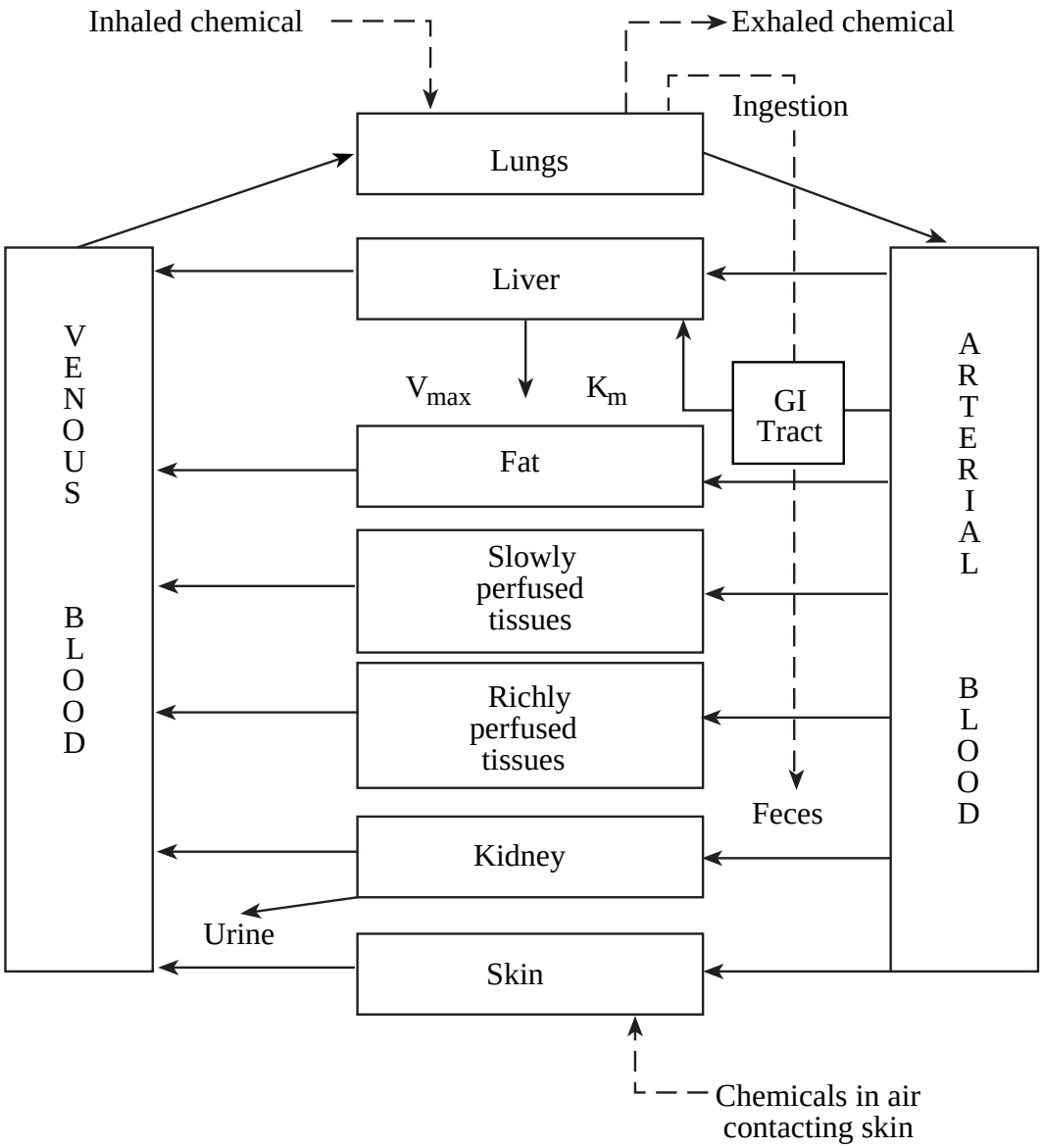
3.5 MECHANISMS OF ACTION

3.5.1 Pharmacokinetic Mechanisms

Absorption. No studies were located regarding the mechanism of absorption of atrazine in humans or animals by any route.

Atrazine is only slightly soluble in water, but has a fairly high solubility in *n*-octanol, with an octanol/water partition coefficient of 322 (Balke and Price 1988). Examination of the interaction of atrazine with 1,2-dipalmitoyl-3-*sn*-phosphatidylcholine (DPPC), a model for biological membranes, showed that atrazine does not perturb the hydrophobic core of the lipid bilayer, but localizes superficially near the

Figure 3-2. Conceptual Representation of a Physiologically Based Pharmacokinetic (PBPK) Model for a Hypothetical Chemical Substance



Source: adapted from Krishnan et al. 1994

Note: This is a conceptual representation of a physiologically based pharmacokinetic (PBPK) model for a hypothetical chemical substance. The chemical substance is shown to be absorbed via the skin, by inhalation, or by ingestion, metabolized in the liver, and excreted in the urine or by exhalation.

glycerol backbone (Tanfani et al. 1990). This does not seem to support passive diffusion through the gastrointestinal tract or skin.

Distribution. No studies were located regarding the mechanism of distribution of atrazine in humans or animals by any route.

Once absorbed, atrazine is transported throughout the body in the plasma (Timchalk et al. 1990). Atrazine has been detected in the kidney, small intestine, lung, liver, pancreas, muscle, heart, and plasma of a man who ingested weedkiller that contained atrazine (Pommery et al. 1993).

Metabolism. Atrazine is metabolized to its mono-dealkylated derivatives, and maybe to didealkylated atrazine, in humans (Ademola et al. 1993; Buchholz et al. 1999; Catenacci et al. 1993) and animals (Bakke et al. 1972, Gojmerac and Kniewald 1989; Meli et al. 1992; Timchalk et al. 1990). *In vitro* studies using microsomal preparations from liver and other tissues of humans and animals have indicated that dealkylation of atrazine is mediated by cytochrome P-450 enzymes and requires NADPH (Adams et al. 1990; Ademola et al. 1993; Croce et al. 1996; Hanioka et al. 1998a, 1999; Lang et al. 1996, 1997; Meli et al. 1992; Venkatesh et al. 1992). Additional *in vitro* studies have indicated that CYP1A2, 2C19, and 1A1 may be the primary metabolic enzymes for atrazine in humans (Lang et al. 1997), while CYP2B1 and 2C11 may be the primary CYPs responsible for atrazine metabolism in rats (Hanioka et al. 1998a). Thus, some species-specificity regarding phase I metabolism of atrazine is evident.

Atrazine also reportedly undergoes phase II metabolism, involving glutathione conjugation and conversion to mercapturic acid derivatives (Adams et al. 1990; Egaas et al. 1995).

Excretion. Atrazine is excreted as dealkylated and mercapturic acid derivatives primarily in the urine (Bakke et al. 1972; Buchholz et al. 1999; Catenacci et al. 1990, 1993; Meli et al. 1992; Timchalk et al. 1990), with feces being a minor route of excretion (Buchholz et al. 1999; Timchalk et al. 1990).

3.5.2 Mechanisms of Toxicity

The primary target of atrazine in some animal species is the female reproductive system. Altered estrus cyclicity has been observed in Sprague-Dawley, Long-Evans, and Donryu rats following exposure to $5 mg/kg/day atrazine for intermediate or chronic durations (Aso et al. 2000; Cooper et al. 1996b; Eldridge et al. 1994a, 1999a; Wetzel et al. 1994) and to a single dose of 300 mg/kg/day (Cooper et al.

2000). These effects do not appear to be the result of intrinsic estrogenic activity of atrazine. Aso et al. (2000) found no increases in BrdU-positive (dividing) cells in the uterus of Sprague-Dawley, Long-Evans, or Donryu rats following 28 days of oral exposure to up to 50 mg/kg/day atrazine. Sprague-Dawley rats that received up to 300 mg/kg/day orally for 3 days had no increases in uterine weight, cytosolic progesterone receptor binding, or peroxidase activity; positive controls that received 17β-estradiol had increases in all three parameters (Connor et al. 1996). Tennant et al. (1994b) also found no increase in uterine weight in Sprague-Dawley rats exposed to 300 mg/kg/day for 3 days, supporting a lack of estrogenic activity. A recent set of experiments has indicated that atrazine may disrupt endocrine function, and the estrus cycle, primarily through its action on the central nervous system (Cooper et al. 2000) in a manner very similar to the known mechanism of reproductive senescence in some strains of rats. In certain strains of rats, including Sprague-Dawley and Long-Evans, reproductive senescence begins by 1 year of age, and results from inadequate stimulation of the pituitary by the hypothalamus to release LH; low serum levels of LH leads to anovulation, persistent high plasma levels of estrogen, and persistent estrus. Atrazine apparently accelerates the process of reproductive senescence in these strains of rats.

Atrazine has been shown to induce mammary tumor formation in female Sprague-Dawley rats, but not male Sprague-Dawley or male or female Fischer-344 rats (Wetzel et al. 1994). This effect is also thought to be the result of acceleration of reproductive senescence, as described above. Failure to ovulate and a state of persistent estrus leads to constant elevated serum levels of endogenous estrogen, which may result in tumor formation in estrogen-sensitive tissues. Therefore, the mechanism of disruption of normal reproductive cyclicity and mammary carcinogenicity in these strains of rat likely does not involve direct interaction of atrazine with estrogen or the estrogen receptor. It also is probably not an adequate model for human reproductive toxicity or carcinogenicity because reproductive senescence in women involves ovarian depletion and decreased serum estrogen levels instead of decreasing hypothalamic function and increased serum estrogen levels (Carr 1992).

As previously stated, atrazine has been shown to alter serum luteinizing hormone (LH) and prolactin levels in Sprague-Dawley rats by altering the hypothalamic control of these hormones (Cooper et al. 2000). LH and prolactin are released from the pituitary in response to gonadotropin-releasing hormone (GnRH) from the hypothalamus, which is regulated by the interactions of various ligands with the gamma-aminobutyric acid receptor ($GABA_A$ receptor). Shafer et al. (1999) examined the effect of atrazine and other triazine herbicides on $GABA_A$ receptors in cortical tissue from rat brain and found that atrazine can interfere with the binding of some ligands, but not others, to the $GABA_A$ receptor in a

noncompetitive manner. The mono- and didealkylated atrazine metabolites had no effect on $GABA_A$ receptor binding. These preliminary data support the hypothesis that the hormonal effects of atrazine in Sprague-Dawley rats may be mediated through the $GABA_A$ receptor in the central nervous system. Although the effects of atrazine interaction with $GABA_A$ receptors on reproductive senescence may be peculiar to a few strains of rats, atrazine interaction with $GABA_A$ receptors may occur in other rat strains and in other species, including humans, with effects not yet realized. No data are currently available regarding this mechanism in humans.

Sanderson et al. (1999) has demonstrated that atrazine and its two primary metabolites, deethyl- and deisopropylatrazine, are capable of inducing aromatase (CYP19) activity, with a corresponding increase in aromatase ribonucleic acid (RNA), in the human adrenocortical carcinoma cell line, H195R. Aromatase is the rate-limiting enzyme in the conversion of androgens to estrogens, and its induction could play a role in estrogen-mediated pathologies. Atrazine has also been shown to alter the ratio of metabolites of estradiol in the estrogen receptor-positive (ER+) human breast cell line, MCF-7 (Bradlow et al. 1995). Estradiol metabolism proceeds via hydroxylation at one of two mutually exclusive carbons, C-2 or C-16α. The C-2 product, $2\text{-}OHE_1$, is much less potent than estradiol (and may even be anti-estrogenic) and is nongenotoxic. The C-16α product, $16\alpha\text{-}OHE_1$, is a fully potent estrogen that is genotoxic, tumorigenic, and causes increased cell proliferation by covalently binding to estrogen receptors and interacting with deoxyribonucleic acid (DNA). The ratio of $16\alpha\text{-}OHE_1/2\text{-}OHE_1$ in MCF-7 cells incubated with atrazine was approximately 12 times that of untreated control cells, and was several times that of cells treated with DMBA, a known carcinogen. Atrazine caused both a decrease in the amount of $2\text{-}OHE_1$ and an increase in the amount of $16\alpha\text{-}OHE_1$. These data suggest that atrazine could play a role in cancer development in estrogen-responsive tissues, since studies have shown that an elevated $16\alpha\text{-}OHE_1/2\text{-}OHE_1$ ratio is associated with breast and other cancers in animals (Bradlow et al. 1995; Telang et al. 1992). In similar experiments using the ER^- cell lines, MDA-MB-231 and MCF-10, no inhibitory or stimulatory changes in estrogen metabolism were seen (Bradlow et al. 1997). This suggests that ER status of cells plays a role in the ability of atrazine to cause changes that might result in cancer of estrogen-responsive tissues. It has been speculated that two response elements in the DNA of these cells, one requiring the xenobiotic (atrazine) and one requiring an ER-ligand complex, must be activated in order to initiate an increase in expression of the cytochrome P-450 enzyme responsible for 16α-hydroxylation of estrogen (Bradlow et al. 1997).

Atrazine may also interfere with male hormone regulation and activity. Testosterone conversion to its primary metabolite, 5α-dihydroxytestosterone (5α-DHT), was significantly decreased in rat prostate tissue

exposed to 0.465–1.392 µmol atrazine for three hours (Kniewald et al. 1995). Additionally, the number of receptor binding sites for 5α-DHT were reduced in prostate homogenates from rats that had received 60 or 120 mg/kg/day atrazine orally for 7 days (Kniewald et al. 1995; Šimi*ƒ* et al. 1994). These effects are reversible upon cessation of atrazine exposure, although recovery in prepubescent rats was slower than in adult rats. A detailed mechanism for these effects has not been elucidated.

3.5.3 Animal-to-Human Extrapolations

The most sensitive target of atrazine toxicity in animals is the reproductive system. A number of studies have shown altered estrus cyclicity and plasma hormone levels in rats exposed to 6.9–300 mg/kg/day; some rat strains, especially Sprague-Dawley and Long-Evans, appear to be more sensitive to these effects (Cooper et al. 1996b, 2000; Eldridge et al. 1994a, 1999a; Šimi*ƒ* et al. 1994; Wetzel et al. 1994). These effects are not likely to be mediated by estrogenic activity of atrazine since it has been shown that atrazine does not bind to estrogen receptors *in vitro* or induce uterine decidualization in rats (Aso et al. 2000; Connor et al. 1996; Tennant et al. 1994b). There is some evidence that the estrus cycle effects are the disruption of the gonadal-hypothalamic-pituitary axis, which results in lower GnRH release from the hypothalamus and, ultimately, lack of ovulation increased plasma estradiol levels, and persistent estrus (Cooper et al. 2000). Strains that normally experience reproductive senescence via the same mechanism are more likely to experience estrus disruption in response to atrazine. However, reproductive senescence in humans is characterized by ovarian depletion and decreased estrogen levels, making it unlikely that effects similar to the estrus effects seen in rats would occur in humans. Therefore, the rat does not appear to be an appropriate model for this end point. Shafer et al. (1999) has demonstrated (*in vitro*) that atrazine can inhibit the binding of some, but not all, ligands to the GABA receptor. The GABA receptor-ligand complex acts on $GABA_A$ chloride channels in the hypothalamus, stimulating the release of GnRH. Inhibition of ligand binding to GABA receptors could contribute to the disruption of the estrus cycle in rats, although this has not been demonstrated *in vivo*. The GABA receptor has many isomeric forms with diverse pharmacology. It is possible that atrazine could interact with the GABA receptor(s) in other species, including humans, with different effects.

3.6 TOXICITIES MEDIATED THROUGH THE NEUROENDOCRINE AXIS

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones. Chemicals with this type of activity are most commonly referred to as *endocrine disruptors*. However, appropriate

terminology to describe such effects remains controversial. The terminology *endocrine disruptors*, initially used by Colborn and Thomas (1992) and again by Colborn (1993), was also used in 1996 when Congress mandated the Environmental Protection Agency (EPA) to develop a screening program for "...certain substances [which] may have an effect produced by a naturally occurring estrogen, or other such endocrine effect[s]...". To meet this mandate, EPA convened a panel called the Endocrine Disruptors Screening and Testing Advisory Committee (EDSTAC), which in 1998 completed its deliberations and made recommendations to EPA concerning *endocrine disruptors*. In 1999, the National Academy of Sciences released a report that referred to these same types of chemicals as *hormonally active agents*. The terminology *endocrine modulators* has also been used to convey the fact that effects caused by such chemicals may not necessarily be adverse. Many scientists agree that chemicals with the ability to disrupt or modulate the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. However, others think that endocrine-active chemicals do not pose a significant health risk, particularly in view of the fact that hormone mimics exist in the natural environment. Examples of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Livingston 1978; Mayr et al. 1992). These chemicals are derived from plants and are similar in structure and action to endogenous estrogen. Although the public health significance and descriptive terminology of substances capable of affecting the endocrine system remains controversial, scientists agree that these chemicals may affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body responsible for maintaining homeostasis, reproduction, development, and/or behavior (EPA 1997). Stated differently, such compounds may cause toxicities that are mediated through the neuroendocrine axis. As a result, these chemicals may play a role in altering, for example, metabolic, sexual, immune, and neurobehavioral function. Such chemicals are also thought to be involved in inducing breast, testicular, and prostate cancers, as well as endometriosis (Berger 1994; Giwercman et al. 1993; Hoel et al. 1992).

There is considerable evidence that atrazine interferes with the normal function of the endocrine system. Increases in pituitary gland weight and enlarged pituitaries have been observed in male and female rats exposed to 12 mg/kg/day atrazine and higher for acute-, intermediate-, and chronic-durations (Babic-Gojmerac et al. 1989; EPA 1984a, 1987a; Šimi*ƒ* et al. 1994). Significant decreases in pituitary hormones have also been observed. Decreases in prolactin and luteinizing hormone levels have been observed in rats exposed for 1, 3, or 21 days (Cooper et al. 2000) or 9 months (Wetzel et al. 1994).

In the reproductive system, these alterations in pituitary hormone levels sometimes result in significant alterations in blood estradiol and progesterone levels (Cooper et al. 1996b; Eldridge et al. 1994a; Wetzel

et al. 1994). Whether these hormone levels are increased or decreased appears to be strain specific in rats, as well as age-related. The alterations in estradiol and progesterone levels can affect estrus cyclicity. Disruption of the percentage of days in estrus or diestrus has been observed Long Evans and Sprague-Dawley (Cooper et al. 1996b, 2000; Eldridge et al. 1994a; Wetzel et al. 1994). In general, reproductive effects have not been identified in males.

The toxicity of atrazine to the pituitary has also resulted in developmental effects. When rat dams were exposed to atrazine during lactational days 1–4, atrazine suppressed the prolactin surge, which is usually induced by pup suckling. The resultant decreased prolactin levels in breast milk, resulted in prostate inflammation in the adult offspring (Stoker et al. 1999).

3.7 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate resulting from maternal exposure during gestation and lactation. Relevant animal and *in vitro* models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in Section 6.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects, and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both prenatal and postnatal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example,

infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Altman and Dittmer 1974; Fomon 1966; Fomon et al. 1982; Owen and Brozek 1966; Widdowson and Dickerson 1964). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testis barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns. At various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults, and sometimes unique enzymes may exist at particular developmental stages (Komori et al. 1990; Leeder and Kearns 1997; NRC 1993; Vieira et al. 1996). Whether differences in xenobiotic metabolism make the child more or less susceptible also depends on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in newborns who all have a low glomerular filtration rate and have not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; NRC 1993; West et al. 1948). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer remaining lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility, whereas others may decrease susceptibility to the same chemical. For example, although infants breathe more air per kilogram of body weight than adults breathe, this difference might be somewhat counterbalanced by their alveoli being less developed, which results in a disproportionately smaller surface area for alveolar absorption (NRC 1993).

There is no direct information on the toxicity of atrazine in children and no information on effects in adults who were exposed as children. Animal data indicate that the primary target of atrazine is the reproductive system and that atrazine can affect adult animals, which may result in effects in the offspring. Male rats exposed to 25, but not 12.5, mg/kg/day atrazine via lactation on postpartum days 1–4 had inflammation of the lateral prostate at 120 days of age (Stoker et al. 1999). This effect was thought to be the result of a lack of prolactin release in the dam in response to pup suckling, which was verified by monitoring plasma prolactin levels during and after pup suckling. Also, co-administration of ovine prolactin with atrazine to the dam eliminated the increase in prostate inflammation in offspring. Prolactin plays an important role in the postnatal development of the tuberoinfundibular dopaminergic (TIDA) system, which in the adult rat has an inhibitory effect on prolactin release from the pituitary (Shyr et al. 1986). A lack of prolactin during development results in a lack of prolactin release control and

hyperprolactinemia in the adult rats, which leads to lateral prostate inflammation (Tangbanluekal and Robinette 1993).

Peruzovif et al. (1995) found subtle neurobehavioral effects (increased spontaneous activity in females and increased performance in avoidance conditioning trials in males) in offspring of rat dams exposed to 120 mg/kg atrazine 6 times during a 12-day period that ended 4 weeks before the rats were bred. The mechanism for this effect is unknown, but since atrazine is not thought to persist in tissues, it may be mediated through changes in the dam that later affect the offspring. These data indicate that the developing organism may be susceptible to the effects of atrazine and/or its metabolites.

There are no studies that indicate that metabolism of atrazine differs between children and adults or between young and adult animals. The primary pathway by which atrazine is metabolized is dealkylation to yield the mono- and/or didealkylated atrazine derivatives. *In vitro* studies with human liver microsomes and recombinant cytochrome P-450 (CYP) isozymes indicate that multiple CYP isozymes are probably involved in the dealkylation of atrazine in humans (Lang et al. 1997). This study indicates that CYP1A2, CYP2C19, and CYP1A1 may be the major CYP enzymes for atrazine, but that other forms, including CYP2A6, CYP2C9, and CYP2B6, are likely to be major contributors, especially in individuals with low levels of CYP2C19 or CYP1A2. While CYP2C19 and CYP1A2 are not present in appreciable levels in human fetal liver, their activities increase to adult levels by 4–6 months of age (Leeder and Kearns 1997; Ratenasavanh et al. 1991; Sonnier and Cresteil 1998). These data indicate that infants, at or shortly after birth, are capable of metabolizing atrazine to its dealkylated metabolites.

No data were located regarding the passage of atrazine or its metabolites across the placenta or its excretion in breast milk.

3.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s) or the product of an interaction between a xenobiotic agent and some target

molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s), or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to atrazine are discussed in Section 3.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by atrazine are discussed in Section 3.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 3.10 “Populations that are Unusually Susceptible”.

3.8.1 Biomarkers Used to Identify or Quantify Exposure to Atrazine

Atrazine is primarily excreted in the urine as dealkylated metabolites and mercapturic acid derivatives (Bakke et al. 1972; Buchholz et al. 1999; Catenacci et al. 1993; Gojmerac and Kniewald 1989; Meli et al. 1992; Timchalk et al. 1990), which can be detected in urine at levels as low as 1 µg/L (Ikonen et al. 1988). The presence of atrazine derivatives, especially the mercapturic acid derivatives, are useful biomarkers of exposure (Jaeger et al. 1998; Lucas et al. 1993); however, atrazine is eliminated from the

body in 24–48 hours (Catenacci et al. 1990; Meli et al. 1992; Timchalk et al. 1990) and thus, the tests must be performed soon after the exposure. Atrazine and its metabolites can also be detected in blood and tissues at levels as low as 14.25 ng/g (Pommery et al. 1993). The detection of atrazine in urine or tissues may be a specific biomarker for atrazine exposure, but <2% of atrazine is excreted in the urine unchanged (Buchholz et al. 1999; Catenacci et al. 1993). The detection of atrazine metabolites is not specific for atrazine exposure, but may also be a biomarker of exposure to other triazine herbicides such as cyprazine, simazine, or propazine (Bradway and Moseman 1982; Hanioka et al. 1999; Larsen and Bakke 1975). Analysis for dealkylated metabolites should be performed soon after sample collection because they can degrade over time and during freezing and thawing (Bradway and Moseman 1982); mercapturic acid derivatives may provide a more reliable biomarker (Jaeger et al. 1998; Lucas et al. 1993). There is no quantitative relationship between exposure levels and levels of atrazine or metabolites found in the body (Lucas et al. 1993). Some of the analytical methods used to detect atrazine in biological samples are provided in Table 7-1.

A pair of studies by Lu et al. (1997, 1998) measured the levels of atrazine in saliva in rats under different blood concentrations of atrazine (regulated by intravenous infusion) and different salivary flow rates (controlled by administration of pilocarpine) and found that salivary atrazine levels reflected the levels of free atrazine in the plasma. No attempt was made to measure atrazine metabolites. Salivary levels of atrazine may be a convenient way to determine exposure, but has not been shown to be quantitatively related to oral or dermal exposure levels.

3.8.2 Biomarkers Used to Characterize Effects Caused by Atrazine

The primary target organs of atrazine are the female reproductive system and the liver. The reproductive effects in animals included altered estrus cyclicity or anestrus (Cooper et al. 1996b, 2000; , uri*f* et al. 1999; Eldridge et al. 1994a, 1999a; Gojmerac et al. 1996, 1999; Šimi*f* et al. 1994; Wetzel et al. 1994), altered serum and/or pituitary hormone levels (Cooper et al. 1996b; Eldridge et al. 1994a; Gojmerac et al. 1996, 1999), reduced fecundity (Šimi*f* et al. 1994), decreased ovarian and uterine weights (Eldridge et al. 1994a), and ovarian histopathology (, uri*f* et al. 1999; Gojmerac et al. 1996). The hepatic effects seen following atrazine exposure were increased serum lipids and liver enzymes (Gojmerac et al. 1995; Morichetti et al. 1992; Radovcic et al. 1978; Santa Maria et al. 1987; Wurth et al. 1982), liver histopathology (, uri*f* et al. 1999; Gojmerac et al. 1995), changes in liver weight (Aso et al. 2000; EPA 1984a, 1987a, 1989), and changes in trigycerides and globulin levels (EPA 1984a, 1987a). While all of these effects may be useful biomarkers to indicate possible atrazine exposure, none are specific for

atrazine. Additionally, it is unclear which, if any, of the above reproductive effects may be caused by atrazine exposure in humans.

3.9 INTERACTIONS WITH OTHER CHEMICALS

No data were located regarding interactions of atrazine with other chemicals in humans. Ugazio et al. (1991a, 1991b, 1993) examined the effects of atrazine on hexabarbital-induced sleep time (HB-ST) in rats. Atrazine exposure consistently reduced HB-ST, especially in males, indicating an induction of microsomal enzymes (Ugazio et al. 1991a). In offspring of treated animals, that received atrazine via lactation and then directly following weaning, HB-ST was also shortened, most notably at weaning (21 days of age). Induction of enzymes was verified by determination of liver microsomal protein concentrations and metabolic enzyme activities in male rats; all were elevated significantly at weaning only, and elevated without statistical significance thereafter (Ugazio et al. 1991a). A single dose of atrazine to Wistar rats also reduced HB-ST and elevated some metabolic enzymes; and atrazine co-administered with carbon tetrachloride (CCl_4) attenuated the effects of CCl_4 (Ugazio et al. 1993). Therefore, atrazine may alter the effects of other chemicals via the induction of metabolic enzymes in the liver.

3.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to atrazine than will most persons exposed to the same level of atrazine in the environment. Reasons may include genetic makeup, age, health and nutritional status, and exposure to other toxic substances (e.g., cigarette smoke). These parameters result in reduced detoxification or excretion of atrazine, or compromised function of organs affected by atrazine. Populations who are at greater risk due to their unusually high exposure to atrazine are discussed in Section 6.7, Populations with Potentially High Exposures.

Few data are available regarding populations that may be unusually susceptible to atrazine. Limited human data suggest that there may be a relationship between elevated levels of atrazine, as well as other pesticides, in drinking water and intrauterine growth retardation (Munger et al. 1997). No individual exposure data were available and a definite causal relationship could not be determined. Developmental effects have been observed in animals and include postimplantation losses, decreases in fetal body weight, incomplete ossification, neurodevelopmental effects, and impaired development of the reproductive system (Infurna et al. 1988; Peruzovi*ƒ* et al. 1995; Stoker et al. 1999). Severe maternal toxicity was noted

at the higher dose levels in some studies. Species differences in sensitivity were noted, with rabbits being substantially more sensitive than rats (Infurna et al. 1988); the relative sensitivity of pregnant humans to atrazine has not been determined. Taken together, these data suggest that it may be prudent to consider the pregnant organism as unusually susceptible to atrazine exposure.

Atrazine has been shown to cause liver effects in animals; therefore, people with liver damage or disease may be at greater risk from exposure to atrazine.

3.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to atrazine. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to atrazine. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following texts provide specific information about treatment following exposures to atrazine:

Ellenhorn MJ, Barceloux DG. 1988. Medical toxicology: Diagnosis and treatment of human poisoning. New York, NY: Elsevier, 1078-1080.

Haddad LM, Winchester JF. 1990. Clinical management of poisoning and drug overdose. Second edition. Philadelphia, PA: W.B. Sanders Company, 1084-1085.

3.11.1 Reducing Peak Absorption Following Exposure

Data regarding the reduction of atrazine absorption in humans after inhalation exposure were not located. Oral absorption of atrazine can be reduced with gastric lavage, activated charcoal, sodium sulfate, and cathartics (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Since many commercial formulations of organochlorine insecticides contain organic solvents, emesis is not usually recommended due to the hazard of solvent aspiration (Ellenhorn and Barceloux 1988). In addition, oils should usually not be used as cathartics since they may enhance the absorption of atrazine (Haddad and Winchester 1990).

Dermal absorption of atrazine can be reduced by removing contaminated clothing and thoroughly washing the exposed skin with a mild soap (Ellenhorn and Barceloux 1988; Haddad and Winchester

1990). Oils should not be used as a cleansing agent since they may facilitate dermal absorption (Haddad and Winchester 1990).

3.11.2 Reducing Body Burden

No experimental data regarding methods for reducing the atrazine body burden were located. Since animal studies indicate that atrazine is rapidly metabolized and cleared from the body, methods for reducing body burden are not expected to be especially effective in reducing human exposures.

3.11.3 Interfering with the Mechanism of Action for Toxic Effects

No reports of methods that would interfere with the mechanism of atrazine toxicity were identified.

3.12 ADEQUACY OF THE DATABASE

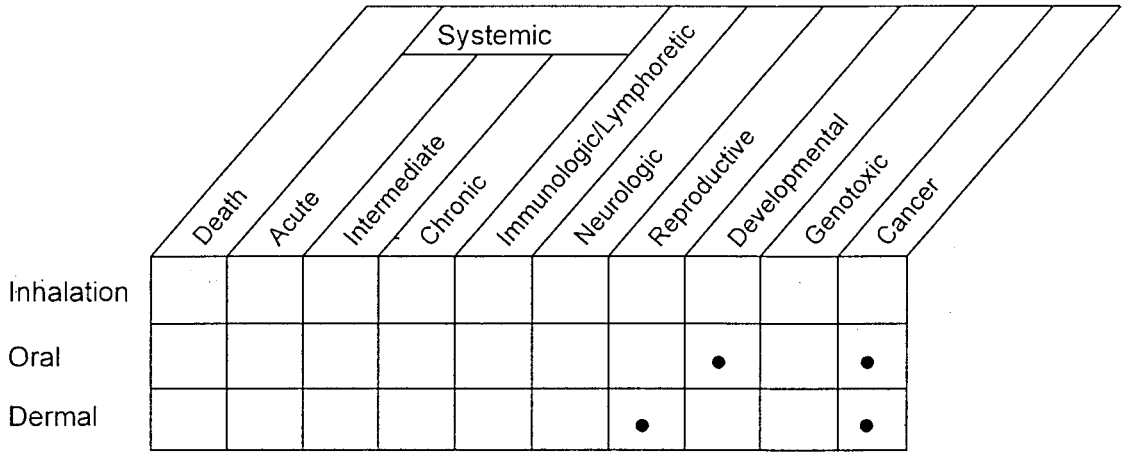
Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of atrazine is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of atrazine.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

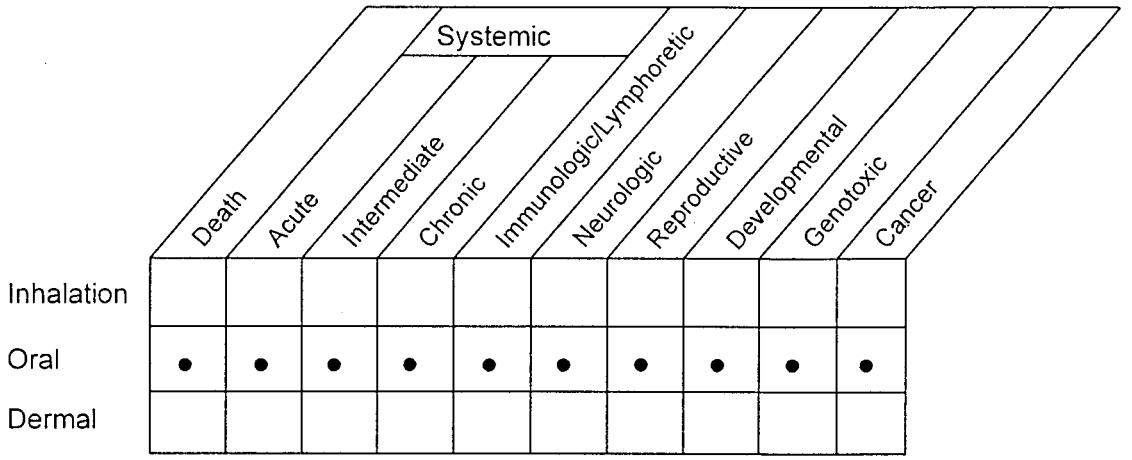
3.12.1 Existing Information on Health Effects of Atrazine

The existing data on health effects of inhalation, oral, and dermal exposure of humans and animals to atrazine are summarized in Figure 3-3. The purpose of this figure is to illustrate the existing information concerning the health effects of atrazine. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the

Figure 3-3. Existing Information on Health Effects of Atrazine



Human



Animal

● Existing Studies

quality of the study or studies, nor should missing information in this figure be interpreted as a "data need". A data need, as defined in ATSDR's *Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles* (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

There are limited data on the toxicity of atrazine in humans. The available ecological studies examined the potential of atrazine to induce reproductive and developmental effects and cancer. Two case reports discuss the lethality of atrazine and its toxic effect to the skin.

The database for health effects of atrazine in laboratory animals is limited to oral studies, as can be seen in Figure 3-3. These studies have examined lethality, systemic, reproductive, and developmental toxicity, and carcinogenicity. Although some studies have examined the immunotoxicity and neurotoxicity of atrazine, these potential effects have not been thoroughly investigated. Genotoxicity data on atrazine are available from both *in vitro* and *in vivo* studies.

3.12.2 Identification of Data Needs

Acute-Duration Exposure. The only human data on the acute toxicity of atrazine are two case reports, that describe the lethality (Pommery et al. 1993) and the dermal toxicity (Schlicher and Beat 1972). Because each report only described one individual, interpretation of the study is limited. Studies in laboratory animals are limited to oral exposure. Acute-duration oral studies in animals primarily focused on the endocrine and reproductive toxicity of the compound. These studies reported alterations in pituitary weight or size (Babic-Gojmerac et al. 1989; Šimi*ƒ* et al. 1994), thyroid gland histology and thyroid hormone levels (Kornilovskaya et al. 1996), pituitary hormone levels (Cooper et al. 2000), and effects on the estrus cycle (Cooper et al. 2000; Šimi*ƒ* et al. 1994). The developmental toxicity of atrazine has also been investigated in several studies that found profound maternal toxicity in rats and rabbits (Infurna et al. 1988), less severe skeletal effects (incomplete ossification) (Infurna et al. 1988), prostatitis in male offsprings (Stoker et al. 1999), and neurodevelopmental effects (Peruzovi*ƒ* et al. 1995). With the exception of endocrine and body weight effects, most of the acute-duration studies did not examine for systemic effects. A study by Santa Maria et al. (1987) did report renal and hepatic effects. Additional oral studies are needed to establish dose-response relationships for endocrine effects, which appears to be the most sensitive target of toxicity. Inhalation and dermal exposure studies are needed to identify the critical effect for these routes and establish dose-response relationships.

Intermediate-Duration Exposure. No human studies involving intermediate-duration exposure to atrazine were located. Additionally, no animals inhalation or dermal exposure studies were identified. As with acute toxicity, the intermediate-duration studies primarily focused on the ability of atrazine to disrupt the endocrine system and alter the estrus cycle. A number of studies have examined hormone levels and the estrus cycle in several strains of rats exposed to atrazine (Aso et al. 2000; Cooper et al. 2000; Eldridge et al. 1994a; Wetzel et al. 1994). These studies also reported decreases in body weight gain. Studies in pigs (, uri*f* et al. 1999; Gojmerac et al. 1995, 1996, 1999) have examined reproductive and systemic end points and reported very low LOAEL values. None of the other available studies examined a wide range of potential systemic effects. Additional oral studies that examine the potential systemic toxicity of atrazine are needed. Inhalation and dermal exposure studies are also needed to identify critical effects and establish dose-response relationships.

Chronic-Duration Exposure and Cancer. Ecological studies designed to assess the reproductive toxicity (Curtis et al. 1999; Savitz et al. 1997) following dermal and inhalation exposure and developmental toxicity following oral exposure (Munger et al. 1999) have been identified. Several studies have investigated the chronic toxicity of atrazine following oral exposure of laboratory animals. Studies in rats (EPA 1984a, 1987a) and dogs (EPA 1989) have reported decreased erythrocyte parameters, liver effects, functional impairment of the kidney (rats only), cardiac effects (dogs only), endocrine effects (enlarged pituitary and increased adrenal gland weight; rats only), and decreased body weight gain. The reproductive toxicity of atrazine has also been investigated in rats (Wetzel et al. 1994). Additional oral studies are needed to further define the dose-response relationships. Inhalation and dermal exposure studies are needed to identify critical effects and establish dose-response relationships.

A study of residents drinking water contaminated with atrazine found a significant association between atrazine levels and increased risk of stomach cancer and decreased risk of colon cancer (Van Leeuwen et al. 1999). No other human carcinogenicity data were identified. Oral exposure studies in rats found inconsistent results. An increase in uterine adenocarcinomas was found in one study of female Fischer-344 rats (Pintér et al. 1990), whereas another study did not find any significant increases in tumor incidence in female Fischer-344 rats receiving a similar dose level (Wetzel et al. 1994). The Wetzel et al. (1994) study found a significant increase in mammary and pituitary tumors in female Sprague-Dawley rats. Additional carcinogenicity studies are needed by the inhalation, oral, and dermal routes to better assess the carcinogenic potential of atrazine.

Genotoxicity. The available genotoxicity data indicate that atrazine may have genotoxic potential. *In vivo* genotoxicity studies have found increases in DNA strand breaks (Pino et al. 1988) and micronucleus formation (Gebel et al. 1997) in mice and somatic mutations (Torres et al. 1992; Tripathy et al. 1993), dominant lethal mutations (Murnick and Nash 1977), and aneuploidy (Murnick and Nash 1977) in *Drosophila melanogaster*. In *in vitro* assays using human lymphocytes, atrazine-induced DNA damage (Ribas et al. 1995) and chromosomal aberrations (Meisner et al. 1992, 1993). In general, genotoxic potential was not detected in assays using *S. typhimurium* (Adler 1980; Andersen et al. 1972; Butler and Hoagland 1989; Morichetti et al. 1992; Ruiz and Marzin 1997; Seiler 1973; Zeiger et al. 1988), *E. coli* (Adler 1980; Ruiz and Marzin 1997), or bacteriophages (Andersen et al. 1972). In contrast, studies for gene mutations (Emnova et al. 1987; Mathias et al. 1989; Morichetti et al. 1992; Plewa and Gentile 1976), mitotic recombination (Adler 1980), anaeuploidy (Benigni et al. 1979), and micronucleus formation (Mohammed and Ma 1999) in yeast have been positive. The small number of *in vivo* genotoxicity studies and the apparent conflict between prokaryotic and eukaryotic genotoxicity assay suggest that additional information is needed to assess the genotoxicity of atrazine.

Reproductive Toxicity. The reproductive toxicity of atrazine has been examined in humans exposed via inhalation and dermal exposure and orally exposed animals. In studies of couples living on farms using atrazine, a significant association between herbicide activity and increase in preterm deliveries was seen (Savitz et al. 1997). No association was found with atrazine use and the risk of miscarriages (Savitz et al. 1997) or decreased fecunity (Curtis et al. 1999). Oral exposure studies in rats and pigs have demonstrated that atrazine is a reproductive toxicant. In rats, alterations in estrus cycle (Aso et al. 2000; Cooper et al. 1996b; Eldridge et al. 1994a; Šimi*f* et al. 1994; Wetzel et al. 1994), impaired fertility when exposed females were mated with exposed or unexposed males (Šimi*f* et al. 1994), decreased uterine and ovarian weights (Eldridge et al. 1994a), and decreased serum estradiol levels (Cooper et al. 2000; Eldridge et al. 1994a) were observed. Many of the rat studies tested several rat strains and found significant strain differences. For example, an increase in the number of days in estrus was found in Sprague-Dawley rats, but in Fischer-344 rats, there was a decrease in the percentage of number of days in estrus and an increase in the percentage of days in diestrus (Aso et al. 2000). In pigs, a decrease in serum estrogen levels, increase in serum progesterone levels, absence of estrus onset, multiple ovarian follicular cysts, persisting corpus luteum, and cystic degeneration of secondary follicles were observed (, uri*f* et al. 1999; Gojmerac et al. 1996, 1999).

The rat studies found substantial strain differences and it is not known which rat strain, if any, would be an appropriate model for human reproductive toxicity. Additional studies are needed to address the

apparent strain difference. Reproductive toxicity studies in other species would also address the issue of a model for human reproductive toxicity. The studies by Šimi*ƒ* et al. (1994) in which treated males were mated with untreated females, and the rat 2-generation (EPA 1987b) study are the only available studies that attempted to assess male reproductive toxicity. Šimi*ƒ* et al. (1994) observed a decrease in the number of sperm positive females when atrazine-exposed male and female rats were mated; no effect was seen when exposed males were mated with unexposed females and only a slight effect (82% sperm positive versus 100% in controls) was seen when exposed females were mated with unexposed males. EPA (1987b) found no significant alterations in fertility in a 2-generation rat study in which male and female Charles River albino rats were fed 26.7 mg/kg/day atrazine for at least 10 weeks prior to mating. Additional studies are needed to assess whether the testes is also a sensitive target of atrazine toxicity.

Developmental Toxicity. There are limited data on the developmental toxicity of atrazine in humans. A significant increase in the risk of intrauterine growth retardation was found in a community drinking water contaminated with atrazine (Munger et al. 1997). As with most ecological studies, this study can not establish a definite causal relationship. Developmental toxicity studies in animals are limited to the oral route. In a study (Infurna et al. 1988) of rats (Crl:COBS CD [SD] BR) and rabbits (New Zealand White) exposed to atrazine during gestation, an increase in postimplantation loss was observed in rats and increases in resorptions and postimplantation losses and decreases in live fetuses and fetal body weight were observed in rabbits. However, these fetal effects were accompanied by severe maternal body weight loss and general toxicity. Thus, it is not known if the effects were due to direct toxicity of atrazine to the fetuses or due to atrazine-induced maternal toxicity. For the rats, less severe fetal effects (decreased fetal body weight, incomplete ossification) were observed at the next lowest dose tested and were not associated with severe maternal toxicity. The Infurna et al. (1988) study suggests that the rabbit may be more sensitive that the rat to the toxicity of atrazine, identifying a serious LOAEL at almost the same dose level as a less serious LOAEL in the rat study. Additional developmental toxicity are needed to assess the apparent species differences in developmental toxicity. Rat studies also demonstrated that pregestational exposure to atrazine can result in neurodevelopmental effects in the offspring (Peruzovi*ƒ* et al. 1995) and lactational exposure can result in inflammation of the lateral prostate in adult male offspring (Stoker et al. 1999). Additional studies, particularly studies that examine the offspring as they mature, are needed to further elucidate these effects.

Immunotoxicity. No human studies examining the immunotoxicity of atrazine were located. Oral exposure studies in mice, rats, and pigs suggest that the immune system may be a target of atrazine toxicity. Decreases in thymus weight (Líšková et al. 2000; Vos et al. 1983) and increases in thyroid and mesenteric lymph node weights (Vos et al. 1983) were observed in mice (Líšková et al. 2000) and rats (Vos et al. 1983); lymphoid depletion in the lymphoid follicles of the prescapular and mesenteric lymph nodes were observed in pigs (, uri*f* et al. 1999). The study by Líšková et al. (2000) also included some tests of immune function. Significant alterations in humoral immunity were observed, but no changes in cell-mediated immunity or autoimmunity were found. Additional studies are needed to assess the immunotoxicity of atrazine; a study performing an immunological battery of tests would provide valuable information on the potential of atrazine to impair immune function.

Neurotoxicity. No human data on the neurotoxic potential of atrazine were located. The available data come from two acute-duration oral studies in rats (Bainova et al. 1979; Podda et al. 1997) and an intermediate-duration study in rats (Dési 1983). The acute-duration studies found alterations in cerebellar activity in rats exposed to a moderate dose of atrazine. The intermediate-duration study, tested a slightly lower dose, did not find any differences in a behavioral maze test. These data support the finding of neurodevelopmental effects in the offspring following pregestational exposure (Peruzovi*f* et al. 1995). A neurotoxicity battery is recommended to provide additional information on the neurotoxicity of orally-administered atrazine. Neurotoxicity should also be tested by the inhalation and dermal routes of exposure.

Epidemiological and Human Dosimetry Studies. Limited human cohort and ecological studies have been performed and generally involved exposure to more than one pesticide at poorly-characterized levels during the period of time examined. The primary end points examined included reproductive (Curtis et al. 1999; Savitz et al. 1997), developmental (Munger et al. 1997,) and cancer (Brown et al. 1993; Donna et al. 1989; Mills 1998; Van Leeuwen et al. 1999; Weisenburger 1990; Zahm et al. 1993).

Studies of people occupationally exposed to only atrazine (no other pesticides) would be valuable in assessing the effects of atrazine on human health. Since one of the most significant effects in animals is disruption of estrus cyclicity, epidemiology studies of reproductive parameters in humans exposed to atrazine would be particularly relevant. Such studies would be most valuable if dosimetry methods could be developed to provide reliable exposure data to accompany health effects data. This would assist in establishing cause/effect relationships and in developing methods to monitor individuals living near hazardous waste sites. Such studies are especially necessary because the majority of animal studies

currently available utilize rats, which are not a relevant model for humans for reproductive effects involving disruption of hormonal control of cyclicity and reproductive senescence. Several studies are available that used pigs, with similar results to the rat studies (disruption of estrus cyclicity and/or anestrus); the relevance of pigs as a model for humans for atrazine's effects on hormonal control has not been determined. Studies examining the mechanism of action of atrazine in pigs on estrus cyclicity would be helpful in determining the relevance of pigs as a reproductive model for humans.

Biomarkers of Exposure and Effect.

Exposure. Atrazine is primarily excreted in the urine as dealkylated metabolites and mercapturic acid derivatives (Bakke et al. 1972; Buchholz et al. 1999; Catenacci et al. 1993; Gojmerac and Kniewald 1989; Meli et al. 1992; Timchalk et al. 1990), which can be detected in urine at levels as low as 1 µg/L (Ikonen et al. 1988). Atrazine and its metabolites can also be detected in blood and tissues at levels as low as 14.25 ng/g (Pommery et al. 1993). The detection of atrazine in urine or tissues may be a specific biomarker for atrazine exposure, but <2% of atrazine is excreted in the urine unchanged (Buchholz et al. 1999; Catenacci et al. 1993). The detection of atrazine metabolites is not necessarily specific for atrazine exposure, but may indicate exposure to other triazine herbicides such as cyprazine, simazine, or propazine (Bradway and Moseman 1982; Hanioka et al. 1999; Larsen and Bakke 1975). There is no quantitative relationship between exposure levels and levels of atrazine or metabolites found in the body or in urine (Lucas et al. 1993). Additional studies are needed to establish a relationship between exposure level and urinary concentration of atrazine metabolites.

Effect. The primary target organs of atrazine are the female reproductive system and the liver. The reproductive effects in animals included altered estrus cyclicity or anestrus (Cooper et al. 1996b, 2000; , uri*ƒ* et al. 1999; Eldridge et al. 1994a, 1999a; Gojmerac et al. 1996, 1999; Šimi*ƒ* et al. 1994; Wetzel et al. 1994), altered serum and/or pituitary hormone levels (Cooper et al. 1996b; Eldridge et al. 1994a; Gojmerac et al. 1996, 1999), reduced fecundity (Šimi*ƒ* et al. 1994), decreased ovarian and uterine weights (Eldridge et al. 1994a), and ovarian histopathology (, uri*ƒ* et al. 1999; Gojmerac et al. 1996). The hepatic effects seen following atrazine exposure were increased serum lipids and liver enzymes (Gojmerac et al. 1995; Morichetti et al. 1992; Radovcic et al. 1978; Santa Maria et al. 1987; Wurth et al. 1982), liver histopathology (, uri*ƒ* et al. 1999; Gojmerac et al. 1995), changes in liver weight (Aso et al. 2000; EPA 1984a, 1987a, 1989), and changes in trigycerides and globulin levels (EPA 1984a, 1987a). While all of these effects may be useful biomarkers to indicate possible atrazine exposure, none are specific for atrazine. Additionally, it is unclear which, if any, of the above reproductive effects may occur in humans

following atrazine exposure. Development of additional, more sensitive biomarkers that are specific for atrazine effects would be useful in monitoring populations at high risk. This may need to be done in tandem with the determination of the interaction of atrazine, if any, with the hypothalamus in humans and the elucidation of the mechanism of that interaction.

Absorption, Distribution, Metabolism, and Excretion. The absorption, distribution, metabolism, and excretion of atrazine has been investigated in humans and animals. The only available inhalation toxicity studies in humans involved occupational exposure to very large atrazine particles (30–70 µm) (Catenacci et al. 1990, 1993), which made it unlikely that any significant amount of atrazine reached the lungs. Evidence of absorption following oral exposure was provided by a single case report of a man who ingested a weedkiller containing atrazine and other chemicals; atrazine was detected in the plasma and several organs at autopsy (Pommery et al. 1993). Absorption of atrazine following dermal exposure has been evidenced by the presence of atrazine and its metabolites in urine of people exposed to radiolabelled Aatrex (a commercial product containing atrazine) via a forearm patch (Buchholz et al. 1999), and in urine of workers exposed primarily via dermal contact (Catenacci et al. 1990, 1993). An *in vitro* study using human skin samples also indicated that limited absorption (16.4% in 24 hours) occurs through the skin (Ademola et al. 1993). Further evidence of absorption following oral (Meli et al. 1992; Timchalk et al. 1990) and dermal (Hall et al. 1988) exposure to atrazine has been provided by animal studies showing the presence of atrazine and its metabolites in the plasma, urine, and/or feces. Absorption following gavage administration has been described as a first-order process with an absorption half-life of 2.6 hours (Timchalk et al. 1990), with 37–57% of the administered dose recovered in the urine and 14% in the feces (Meli et al. 1992; Timchalk et al. 1990). Animal studies to determine the absorption efficiency of inhaled atrazine would be useful for determining the risk to occupationally exposed individuals.

Data on distribution of atrazine in humans after oral exposure was limited to a single case report of a 38-year-old man who died of progressive organ failure and shock 3 days after ingesting 500 mL of a weedkiller that contained 100 g atrazine, 25 g of aminotriazole, 25 g of ethylene glycol, and 0.15 g of formaldehyde (Pommery et al. 1993). At autopsy, atrazine was detected in the kidney, small intestine, lung, liver, pancreas, muscle, heart, and plasma. Radioactivity was detected in the plasma, whole skin, and carcass of rats gavaged with 30 mg/kg [C^{14}]-atrazine (Timchalk et al. 1990), and in the liver, brain, heart, lung, kidney, digestive tract, omental fat, and skeletal muscle of rats gavaged with up to 17.7 mg/kg [C^{14}]-atrazine (Bakke et al. 1972). Animal studies to determine the distribution following inhalation and

dermal exposure to atrazine would be useful for evaluating the exposure and risk of occupationally exposed individuals.

Atrazine is extensively and rapidly metabolized as indicated by plasma levels of atrazine and the relative amounts of metabolites and parent compound in the urine within 8–24 hours after exposure. Plasma levels of ^{14}C from radiolabeled atrazine have been shown to peak at 8–10 hours postexposure in rats, and the rate of clearance half-life has been calculated to be 10.8–11.2 hours (Timchalk et al. 1990). In urine, unchanged atrazine has been detected, but comprised <2% of all atrazine-related compounds after dermal exposure in humans (Buchholz et al. 1999; Catenacci et al. 1993) or oral exposure in rats (Meli et al. 1992). In humans, 50% of all urinary atrazine metabolites were excreted within 8 hours and 100% within 24 hours (Catenacci et al. 1993). In rats, approximately 57% of the radioactivity from administered [^{14}C]-atrazine was excreted in the urine within 24 hours (Timchalk et al. 1990), and urinary atrazine metabolites decreased to 1/30 or less of the 24-hour level by 48 hours postexposure (Meli et al. 1992).

Atrazine is primarily metabolized in humans via dealkylation, probably followed by glutathione conjugation and conversion to mercapturic acids. This is apparently true regardless of route of exposure (Buchholz et a. 1999; Catenacci et al. 1993; Meli et al. 1992; Timchalk et al. 1990). *In vitro* studies using microsomal preparations from liver and other tissues of humans and animals have indicated that dealkylation of atrazine is mediated by cytochrome P-450 enzymes (CYPs) (Adams et al. 1990; Ademola et al. 1993; Croce et al. 1996; Hanioka et al. 1998a, 1999; Lang et al. 1996, 1997; Meli et al. 1992; Venkatesh et al. 1992). In humans, the primary CYP responsible for phase I metabolism is probably CYP1A2 (Lang et al. 1997), and in rats, CYPs 2B1 and 2C11 have been implicated as the primary metabolic enzymes (Hanioka et al. 1998a). Available data indicate that phase II metabolism of atrazine proceeds through glutathione conjugation and mercapturic acid formation (Adams et al. 1990; Egaas et al. 1995). Additional studies examining the enzymes responsible for phase I and phase II metabolism and the ratio of products would be useful.

Specific data on elimination and excretion of atrazine by any route were limited. However, the primary route of excretion appears to be in urine, as indicated by the detection of urinary atrazine and its metabolites in a number of species exposed via oral and dermal routes (Bakke et al. 1972; Buchholz et al. 1999; Catenacci et al. 1990, 1993; Meli et al. 1992; Timchalk et al. 1990). Fecal excretion was a minor route (Buchholz et al. 1999; Timchalk et al. 1990). No data were located regarding enterohepatic circulation and biliary secretion or excretion of atrazine in breast milk. Studies to determine whether

enterohepatic circulation occurs and the extent to which it occurs, and studies examining the release of atrazine and its metabolites in breast milk would be helpful in better defining exposure.

Comparative Toxicokinetics. Available data indicate that atrazine is readily absorbed through the intestinal tract (Meli et al. 1992; Pommery et al. 1993; Timchalk et al. 1990) and that limited absorption occurs through the skin (Ademola et al. 1993; Buchholz et al. 1999; Catenacci et al. 1990, 1993; Hall et al. 1988) in humans and animals. Studies examining absorption following inhalation exposure in humans (occupational exposure) and animals would be useful.

Atrazine was detected in the kidney, small intestine, lung, liver, pancreas, muscle, heart, and plasma of a man who ingested weedkiller containing atrazine (Pommery et al. 1993). Radioactivity was detected in the liver, brain, heart, lung, kidney, digestive tract, omental fat, and skeletal muscle of rats gavaged with [C^{14}]-atrazine (Bakke et al. 1972). Additional studies to determine the relative distribution of atrazine and its metabolites in internal organs after inhalation, oral, and dermal exposure to atrazine would be useful. Studies to determine if atrazine crosses the placenta in pregnant animals would also be useful.

While atrazine metabolites have been shown to be qualitatively similar across species, quantitative differences and differences in rate of formation and ratio of products have been observed (Adams et al. 1990; Hanioka et al. 1999; Lang et al. 1996). Inter- and intra-species and age and sex differences in glutathione S-transferase (GST) activity have also been seen (Egaas et al. 1995). Additional studies examining potential sex- and age-related differences between and within species would be useful.

Only 0.3–4.4% of an applied dose of [C^{14}]-atrazine was recovered in urine and 0.0–0.7% in feces of people exposed dermally via an arm patch (Buchholz et al. 1999). No studies were located regarding excretion in humans after oral exposure to atrazine. In rats exposed orally to [C^{14}]-atrazine, 57% of the administered radioactivity was excreted in the urine and only 14% in the feces (Timchalk et al. 1990). Additional studies on routes of elimination of atrazine following exposures of animals by the inhalation, oral, and dermal routes would be useful

Methods for Reducing Toxic Effects. Oral absorption of atrazine can be reduced with gastric lavage, activated charcoal, sodium sulfate, and cathartics (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990); however, animal studies indicate that gastrointestinal absorption of atrazine is fairly rapid (absorption half-life of 2.6 hours) (Timchalk et al. 1990) and thus, these measures would need to be employed soon after exposure. Dermal absorption of atrazine can be reduced by removing contaminated

clothing and thoroughly washing the exposed skin with a mild soap (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990). Additional data regarding interference with gastrointestinal absorption would be useful.

Since animal studies indicate that atrazine is rapidly metabolized and cleared from the body, methods for reducing body burden are not expected to be especially effective in reducing human exposures.

The primary effect of atrazine in rats is disruption of estrus cyclicity, which is mediated through an alteration of the gonadal-hypothalamic-pituitary axis. Differences in reproductive physiology between rats and humans make it unlikely that this mechanism would occur in humans. However, similar effects are seen in pigs and the mechanism has not been elucidated. Additionally, it is not known whether atrazine or its metabolites are responsible for these effects. Studies in pigs and other animals (except rats) to elucidate the mechanism for the reproductive effects of atrazine may be useful for developing methods that can interfere with these effects.

Children's Susceptibility. A single cohort study of farm couples in Canada indicated that atrazine exposure may be associated with increased preterm delivery and miscarriage (Savitz et al. 1997), and an ecological study indicated that atrazine levels in drinking water were positively associated with intrauterine growth rates in the respective communities (Munger et al. 1997). Additional epidemiological studies examining these associations may be useful.

Developmental effects have been observed following pregestational, gestational, and lactational exposure of rat dams to atrazine. The observed effects included postimplantation losses (Infurna et al. 1988), decreases in fetal body weight (Infurna et al. 1988), incomplete ossification (Infurna et al. 1988), neurodevelopmental effects (Peruzovi*f* et al. 1995), and impaired development of the reproductive system (Stoker et al. 1999). A neurodevelopmental toxicity study is needed to verify and further characterize the Peruzovi*f* et al. (1995) results.

There are no studies that indicate that metabolism of atrazine differs between children and adults. The primary pathway by which atrazine is metabolized is dealkylation to yield the mono- and/or didealkylated atrazine derivatives. A study by Lang et al. (1997) indicated that CYP1A2, CYP2C19, and CYP1A1 may be the major CYP enzymes for atrazine, but that other forms, including CYP2A6, CYP2C9, and CYP2B6, are likely to be major contributors, especially in individuals with low levels of CYP2C19 or CYP1A2. While CYP2C19 and CYP1A2 are not present in appreciable levels in human fetal liver, their activities

increase to adult levels by 4–6 months of age (Leeder and Kearns 1997; Ratenasavanh et al. 1991; Sonnier and Cresteil 1998). GST activity, involved in phase II metabolism of atrazine, generally reaches adult levels by 6–18 months of age (Leeder and Kearns 1997). Studies examining the metabolic differences between children and adults may be useful. Studies to determine if atrazine or its metabolites cross the placenta of animals and enter the developing fetus and if they are present in breast milk would also be very useful.

Child health data needs relating to exposure are discussed in Section 6.8.1 Identification of Data Needs: Exposures of Children.

3.12.3 Ongoing Studies

Ongoing studies of atrazine are outlined in Table 3-5 (FEDRIP 2001).

Table 3-5. Ongoing Studies on the Health Effects of Atrazine

Investigator	Affiliation	Research description
Lasley BL	University of California, Davis, California	Methods development for quantification of estrogen receptor- and aryl hydrocarbon receptor- binding xenobiotics
Leszczynski J	Jackson State University, Jackson, Mississippi	Acute toxicity in rats and fish
Tchounwou PB	Jackson State University, Jackson, Mississippi	Toxicokinetics, histopathology, and *in vivo* genotoxicity in rats and fish